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Temporal Changes in Prevalence of *Escherichia coli* Pathotypes in Remote Communities of Ecuador

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HOJA DE APROBACIÓN DE TESIS

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ABSTRACT

Enterotoxigenic *Escherichia coli* (ETEC) is the most prevalent *Escherichia coli* (*E. coli*) pathotype associated with infectious diarrhea in developing countries. However, a previous study found that enteroinvasive *E. coli* (EIEC) was the more prevalent pathotype in 22 remote communities in the northern coastal Ecuador between 2003 and 2005. The purpose of the present study was to determine whether the prominence of this pathotype continued after 2005 and to capture prevalence trends of four diarrheagenic *E. coli* pathotypes between August 2003 and December 2010. The analysis included 4,196 fecal samples collected from 16 remote communities. PCR was used to detect virulence genes of ETEC, EIEC, EPEC, and *E. coli* Shigellae. Our findings suggest that the high prevalence of EIEC between 2005 and 2006 is the result of an outbreak in the 16 communities. In all other years, ETEC was the most prevalent pathotype in the region.

RESUMEN

Escherichia coli enterotoxigénica (ETEC) es la más prevalente de los patotipos de *Escherichia coli* (*E. coli*) asociados con la enfermedad diarreica en los países en vías de desarrollo. Sin embargo, un estudio previo encontró que *E. coli* enteroinvasiva (EIEC) es la más prevalente en 22 comunidades remotas de la Costa norte del Ecuador entre los años 2003 y 2005. El propósito del presente estudio fue comprobar si la prevalencia de este patotipo continuaba después del año 2005 y para determinar la tendencia de la prevalencia de cuatro patotipos de *E. coli* entre Agosto 2003 y Diciembre 2010. El análisis incluyó 4,196 muestras fecales colectadas de 16 comunidades remotas. PCR fue utilizado para detectar los genes de virulencia de ETEC, EIEC, EPEC y *E. coli* Shigellae. Nuestros resultados sugieren que la alta prevalencia de EIEC entre los años 2005 y 2006 es el resultado de un brote en las 16 comunidades. En los otros años, ETEC fue el patotipo más prevalente de la región.

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PART I

GENERAL INTRODUCTION

1. INFECTIOUS DIARRHEA

Infectious diarrhea is one of the most common health problems worldwide; it is the second cause of death (Guerrant et al., 2001; WHO, 2010), principally in children less than 5 years old and is responsible for killing between 1.5 million to 2.5 million children every year in developing countries (WHO, 2010). The main route of transmission is contaminated water or food and affect mainly children who are inmunosupressed, do not have a good diet or have poor hygiene (Kuhnert et al., 2000; Nataro & Kaper, 1998; Lothigius et al., 2008; Okeke, 2009), and death due to fluid and electrolyte loss. Infectious diarrhea is characterized by nausea, vomiting, or abdominal cramps attributable to infectious etiology (Guerrant et al., 2001). In addition, the diarrheal syndromes can be acute watery diarrhea, acute bloody diarrhea, and persistent diarrhea. Acute diarrhea is when the episodes occur at least 14 days, and it is persistent when episodes are for more than 14 days (Sanders & Tribble, 2001; WHO, 2010) although some specialists are considered a third category that is chronic one in where episodes are more than 30 days (Guerrant et al., 2001).

The main etiologic agents associated with diarrhea are viruses, bacteria, and parasites. People might have one or more etiologic agents at the same time (Okeke et al., 2000). First, viruses are the main cause of acute diarrhea in developing countries as well as in developed countries (Rudolph & Cohen, 1999). Viruses-causing diarrhea are principally rotavirus, norovirus, and cytomegalovirus; they account for 70% of the diarrheal cases. In the other hand, among bacterial species that cause diarrhea are *Escherichia coli*, *Vibrio cholera, Campylobacter, Shigella* spp., *Yersinia* spp., *Clostridium difficile,* and *Salmonella* spp. (Rudolph & Cohen, 1999); they are responsible for 20% of diarrheal cases found in children less than five years old. Some parasites such as *Giardia lamblia*, *Cryptosporidium*

spp., and *Entamoeba hystolytica* are implicated with infectious diarrhea and cause near 5% of the cases (Koletzko & Osterrieder, 2009; Qadri et al., 2005).

2. Escherichia coli

Escherichia coli is a Gram-negative, lactose-fermenting bacillus that belongs to the Enterobacteriaceae family (Dedeić -Ljubović et al., 2009, Ishii & Sadowsky, 2008). This species is present in the small and large intestines of warm blooded animals as normal microbiota, and it is also found in the environment (Ishii & Sadowsky, 2008). Intestinal colonization starts after birth and most *Escherichia coli* live as a commensal, in harmony with its host (Kaper et al., 2004; Nataro & Kaper, 1998). At level of environment, this microorganism is found in aquatic ecosystems and where it can resist for a long time (Lothigius et al., 2008; Lothigius et al., 2010). *Escherichia coli* is present in water as a result of human or other animal fecal contamination (Bueno, 2006; Ishii & Sadowsky, 2008, Lothigius et al., 2008; Orsi et al., 2008).

In spite of being commensal, there are some members of this species that cause harm mainly in young animals; and may be more severe in inmunosupressed hosts (Nataro & Kaper, 1998). *Escherichia coli* can cause infections at level of mucosal intestinal or can spread all over the body (Chen & Frankel, 2005); for that reason, *E. coli* is considered as enteric or extra intestinal pathogens (Hernandes et al., 2009; Kaper et al., 2004). The pathogenic traits are responsible for the virulence mechanisms of and the adaptation to different niches (Dedeić-Ljubović et al., 2009; Kaper et al., 2004; Nataro & Kaper, 1998). As a result, they cause three main infections: urinary tract infections, sepsis-meningitis and enteric-diarrheal disease due to its virulence mechanism (Chen & Frankel, 2005; Nataro & Kaper, 1998).

The virulence mechanisms are the result of a combination of virulence factors encoded in bacterial plasmids or chromosomes. The virulence factors in a particular *Escherichia coli* enable it to cause diseases in healthy hosts (Kaper et al., 2004). In order to cause infection, *E. coli* has some strategies like colonization of a mucosal site, avoidance of the host immune system, and intracellular multiplication (Nataro & Kaper, 1998).

According to molecular, pathogenic, and epidemiological features, diarrheagenic *Escherichia coli* (DEC), have been classified in at least seven pathotypes: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohaemorragic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (Kaper et al., 2004; Nataro & Kaper, 1998), and *E. coli* Shigellae (formerly known as *Shigella* spp.) (Lan et al., 2001; Sasakawa, 1997; Yang et al., 2007. In the other hand, some members of DEC share somatic and flagellar antigens, which let to determinate its serotype (Kuhnert et al., 2000; Robins-Browne & Hartland, 2002).

2.1. Enterotoxigenic *Escherichia coli* (ETEC)

Enterotoxigenic *E. coli* (ETEC) is responsible for causing watery diarrhea in animals, infants and young children in developing countries (Kaper et al., 2004; Nataro & Kaper, 1998; Okeke, 2009; Qadri et al., 2005) although ETEC also can cause diarrhea in adult travelers who have visited endemic areas (Kaper et al., 2004; Nataro & Kaper, 1998; Shah et al., 2009). In the other hand, ETEC provoke diarrhea in newborn animals as piglets, lambs, and calves (Kuhnert et al., 2000). In children, ETEC could cause fatal dehydration as a consequence of watery diarrhea, similar to that caused by cholera (Kuhnert et al., 2000; Okeke, 2009; Wolf, 1997).

According to previous studies, ETEC is the most prevalent pathotype in developing countries worldwide (Gupta, et al., 2008; Okeke, 2009; Qadri et al., 2005; WHO, 2010**)**. In Latin America, the diarrhea it is responsible for children mortality (Brüssow et al., 1992; Gómez- Duarte et al., 2010; Paniagua et al., 1997; Rivera et al., 2010). In the other hand, ETEC is one of the most important DEC responsible for around 210 million to 400 million of diarrheal episodes and 380.000 deaths annually among children less than 5 years principally in developing countries (WHO, 2010).

The main route of transmission of ETEC is contaminated food and water (Nataro & Kaper, 1998; Levine, 1987; Okeke, 2009; Qadri et al., 2005). In addition, ETEC has been implicated in water-borne outbreaks (Brüssow et al., 1992; Daniels et al., 2000; Huerta et al., 2000), and in undercooked seafood (Herrera et al., 2010; Jain et al., 2008; Kumar et al., 2005).

ETEC cause diarrhea due the presence of virulence mechanism like colonization factors and enterotoxins, which are encoded in plasmids (Qadri et al., 2005). The presence of colonization factors like adhesive fimbria allows ETEC attachment and colonization of the small intestine (Okeke, 2009). After colonization and multiplication, the enterotoxins stimulate a discharge of electrolytes and water into the lumen causing diarrhea (Cabilio, 2000).

ETEC express two kind of toxins: heat-labile toxin (LT) and heat-stable toxin (ST), both encoded by a plasmid; however, there are some strains of ETEC that may express only LT, only ST or both LT and ST (Kaper et al., 2004; Qadri et al., 2005). The heat-labile toxin (LT) is similar to the cholera toxin in structure and function (Okeke, 2009), LT has two subunits: A which is surrounded by five B subunits (Qadri et al., 2005) and there are two types of LTs: LT-1 encoded by a plasmid and LT-II encode by chromosomal

genes; but only LT-1 is implied in causing diseases in humans. On the other hand, the heat stable toxin (ST) is a peptide of low molecular weight and there are two types: STh discovered in humans and STp found in porcine both of them encoded by a plasmid (Okeke, 2009; Qadri et al., 2005). Both types of STs toxins are related to human infections in childhood than LT strains (Okeke, 2009). Additionally, ETEC carrying ST toxin genes is the most commonly associated with diarrhea (Albert et al., 1999; Qadri et al., 2000; Shaheen et al., 2004), and ST toxin cause more severe disease than LT (Gupta et al., 2008; Qadri et al., 2005).

2.2. Enteropathogenic *Escherichia coli* (EPEC)

Enteropathogenic *E. coli* was first described in United Kingdom in 1945 while studying diarrhea in young children. This is the second most important pathotype of *E. coli,* and causes fatal diarrhea in very young children less than one year old in developing countries (Kaper et al., 2004; Nataro & Kaper, 1998). There are not reports that EPEC affecting travelers and EPEC is not a big problem in developed countries (Trabulsi et al., 2002); however, there are a few cases of diarrhea in adults caused by EPEC due to the ingestion of large inoculum. The principal mechanism of transmission is fecal oral (Nataro & Kaper, 1998).

The principal mechanism of pathogenesis is the damage of intestinal epithelium, attaching and effacing (A/E), characterized by a serious alteration to the of the enterocyte's cytoskeleton (Frankel & Phillips, 2008; Trabulsi et al., 2002). In the damage involves attachment, activation of signal transduction pathways and rearrangements of cytoskeletal proteins, which is caused by virulence factors encoded by a plasmid EAF and the pathogenic island (Frankel & Phillips, 2008; Nataro & Kaper, 1998; Nougayrède et al., 2003). The plasmid EAF encoded a fimbria called bundle-forming pilus (*bfp*); in the other

hand, in the pathogenic island is the locus of enterocyte effacement (LEE) in which there are genes that encoded a type III secretion system, secreted proteins, intimin, and intimin receptor (Tir) (Hernandes et al., 2009; Nataro & Kaper, 1998; Nougayrède et al., 2003; Ochoa et al., 2008; Trabulsi et al., 2002). Its mechanism of action starts with adherence to the intestinal epithelium mediated by the fimbria *bfp* and the intimin, a protein encoded by the chromosomal gene *eae* (Donnenberg & Kaper, 1992; Nougayrède et al., 2003).

The type III secretion system secretes and injects virulence determinants and proteins from the bacteria cytoplasm into the host cells (Robins-Browne & Hartland, 2002). EPEC introduces secreted proteins (Esp) such as EspA, B, and D, which are involved in the construction of translocon. The translocon transports effector molecules to the host cell causing a disorder in the hosts' cytoskeleton (Trabulsi et al., 2002). The Esp A protein form a deep duct that join the bacteria with a host cell while Esp B and D form a pore into the host cell membrane which allows to express the receptor of intimin (Tir) (Robins & Hartland, 2002). The Tir is a translocated protein which is inserted into the host membrane, exposing its middle segment at the cell surface where act as a receptor of intimin (Hernandes et al., 2009; Okeke, 2009; Trabulsi et al., 2002). Once inside of the host cell, intimin moves to the enterocyte membrane and plays an essential role in adhesion and cytoskeletal reorganization (Hernandes et al., 2009; Trabulsi et al., 2002) when binding to the Tir protein. The reorganization of cytoskeletal begin with a flow of signaling events which conduce to a nucleation of cytoeskeletal proteins, altering the cell morphology which let the forming of the attached-effacing lesions (Robins-Browne & Hartland, 2002).

According to the presence or absence of the plasmid EAF, EPECs are divided in typical and atypical (Frankel & Phillips, 2008; Trabulsi et al., 2002). EPEC is typical when it has a chromosomal *eae* gene and EAF in plasmid while EPEC is atypical when has only

the chromosomal *eae* gene but does not possess the plasmid EAF (Hernandes et al., 2009; Nougayrède et al., 2003; Trabulsi et al., 2002). The reservoirs of atypical EPEC are different animal species while typical EPEC has its reservoir only in humans (Trabulsi et al., 2002). EPEC has been considered as a main responsible of persistent diarrhea in children less than 1 year old in developing countries for many years. However, recent evidence suggests that atypical EPEC are more prevalent than typical in developing and developed countries, and may have an important role in persistent diarrhea (Hernandes et al., 2009; Ochoa et al., 2008; Scaletsky et al., 2009; Trabulsi et al., 2002).

2.3. Enteroinvasive *Escherichia coli* (EIEC)

Enteroinvasive *E. coli* is one of the pathotype of *E. coli* implicated to invasive gastrointestinal infections, watery diarrhea, and dysentery in primates including humans as well as *E. coli* Shigellae (Kaper et al., 2004; Jennison & Verma, 2004; Parsot, 2005; Sansonetti, 2001; Schroeder & Hilbi, 2008). *E. coli* Shigellae known as *Shigella* is considered as a variant of enteroinvasive *E. coli* since both microorganisms share molecular and pathogenic properties as a consequence evolutionary convergence (Kaper et al., 2004; Okeke, 2009; Pupo et al., 2000). In other words, both microorganisms share over 90% chromosomal homology and the same virulence plasmid; for that reason, *E. coli* Shigellae is considered inside of *E. coli* species based on molecular data (Lan et al., 2001; Sasakawa, 1997; Yang et al., 2007). In addition, latest phylogenetic studies suggest that *E. coli* Shigellae and EIEC constitute an only pathotype (Lan et al., 2001; Yang et al., 2005).

EIEC and *E. coli* Shigellae are the most important responsible of dysentery in children less than 5 years old and adults (Niyogi, 2005). Dysentery is one of the diseases that constitute an important threat to human health (Sansonetti, 2001; Schroeder & Hilbi, 2008; Wang et al., 2006) because its transmission is mediated by the ingestion of a few amounts

of bacteria and it can be mediated by person to person or by ingestion of contaminated water or food (Jennison & Verma, 2004; Niyogi, 2005; Sasakawa, 1997; Schroeder & Hilbi, 2008). This disease is endemic in developing countries around the world; for that reason, EIEC and *E. coli* Shigellae can be found in endemic areas in which are the common diarrheagenic agent in human (Echeverria et al., 1992a; Faundez et al., 1988; Jennison & Verma, 2004; Taylor et al., 1988). In the other hand, EIEC it can be had a high prevalence in remote areas (Vieira et al., 2007) or it can cause of outbreaks or sporadic cases mainly in regions with poor sanitary conditions (Beutin et al., 1997; Gordillo et al., 1992; Kuhnert et al., 2000).

The pathogenesis of EIEC and *E. coli* Shigellae is the invasion of the epithelium gastrointestinal causing a destruction of the epithelium as a consequence of an inflammatory response (Kaper et al., 2004; Niyogi, 2005; Parsot, 2005). EIEC and *E. coli* Shigellae have an invasion plasmid (pInv), which has about 210 – 230 kb and encoded several virulence factors that allow it to the invasion cellular and its life at level intracellular (Jennison & Verma, 2004; Parsot, 2005; Sasakawa, 2010; Schroeder & Hilbi, 2008). In the other hand, there are three pathogenesis islands (SHI) located in the bacterial chromosome that encoded virulence factor such as a lipopolysaccharide (LPS), enteotoxins (ShET1), proteases, and it has genes that control the expression of virulence genes on the plasmid (Jennison & Verma, 2004; Schroeder & Hilbi, 2008).

The plasmid encodes a type III secretion system, invasion plasmid antigen effectors, and outer membrane protein (Parsot, 2005). The type III secretion system is the principal virulence factor due to it produces the invasion protein antigens (IpaA, ipaB, ipaC, and ipaD) that are effectors of invasion cellular interfering with the cellular process (Jennison & Verma, 2004; Parsot, 2005; Schroeder & Hilbi, 2008). The ipa proteins involved in

invasion cellular are A, and C, while ipaD is associated with cellular invasion and host-cell survival. The ipaB is the principal responsible of macrophage apoptosis and cell-cycle arrest (Ogawa et al., 2008). There is another Ipa protein that is encoded in a different place it is *ipaH*, which is associated with suppression of innate response and liberation of the endocytic vacuoles of phagocyte (Ogawa et al., 2008; Sasakawa, 2010). All ipa proteins are encoded by the *ipa* operon while the *mxi-spa* encoded the structural components of the type III secretion systems that help to deliverer the ipa proteins (Jennison & Verma, 2004; Parsot, 2005). Once the bacteria is in the interior of the host cells, an outer membrane protein, IcsA, drives the nucleation of actin filaments, as a result, the microorganism can move into the cell and the one cell to the another (Bernardini et al.,1989; Jennison & Verma, 2004; Parsot, 2005; Ogawa & Sasakawa, 2006).

For epidemiological purposes, *E. coli* Shigellae can be divided in four groups: *E. coli* Shigellae dysenteriae, *E.coli* Shigellae flexneri, *E. coli* Shigellae boydii, and *E. coli* Shigellae sonnei (Escobar-Páramo et al., 2003; Parsot, 2005; Sasakawa, 1997; Sansonetti, 2001). Those groups can be sub-divided in several serotypes *E. coli* Shigellae dysenteriae, flexneri and boydii, except *E. coli* Shigellae sonnei that has one serotype (Escobar-Paramo et al., 2003). The most important is *E. coli* Shigellae dysenteriae that has a phage borne shiga toxin that is a neurotoxin, enterotoxin, and cytotoxic (Niyogi, 2005) which can cause haemolytic uraemic syndrome (Philpott et al., 2000; Taylor, 2008; Torres, 2004). *E. coli* Shigellae flexneri is mainly responsible for diarrhea in children fewer five years, in developing countries and it could be hyperendemic (Sansonetti, 2001). Only *E. coli* Shigellae flexneri 2a encodes shigella enterotoxin 1 (SHET-1) in its chromosome (Niyogi, 2005; Torres, 2004). *E. coli* Shigellae sonnei is found in developed countries, in contrast of *E. coli* Shigellae boydii, found only in the Indian subcontinent (Niyogi, 2005; Sansonetti,

2001; Torres, 2004). Additionally, the majority of strains of *E. coli* Shigellae produce shigella enterotoxin 2 (SHET2) that is encoded in the large plasmid associated with the virulence pattern of *E. coli* Shigellae and EIEC (Niyogi, 2005).

EIEC is transmitted by food manipulation and person-to-person contact (Harris et al., 1985), an unusual route for ETEC (Daniels et al., 2000; Hunter, 2003; MacDonald et al., 1985). Additionally, EIEC and *E. coli* Shigellae are more sensitive to environmental pH fluctuations and environmental microbiota (Nwachuku & Gerba, 2008; Rosak & Cowell, 1987) and compete poorly with other bacteria (Alcoba-Flórez et al., 2005; Faundez et al., 1988). However, it has been reported that *E. coli* Shigellae can survive for up 6 months in aquatic environments (Khalil et al., 1998; Rahman et al., 1994).

2.4. Enterohaemorragic *Escherichia coli* (EHEC)

Enterohaemorragic *E. coli* belongs to the group of pathogenic strains called Shiga toxin producing *E. coli* (STEC) (Donnenberg & Whittam, 2001; Yoon & Hovde, 2008) due to EHEC express the Stx toxins; in contrast, the other Shiga toxin producing *E. coli*, EHEC has the locus of enterocyte effacement (LEE) (Kaper et al., 2004). EHEC was recognized as human pathogen in 1982 in United States during outbreaks of diarrheal disease associated to the consumption of uncooked beef (Donnenberg & Whittam, 2001; Frankel et al., 1998; Kaper et al., 2004; Robins-Browne & Hartland, 2002). Those outbreaks were linked mainly with ingestion of food or water contaminated mainly with bovine feces (Kaper et al., 2004; Yoon & Hovde, 2008; Lim et al., 2010) because cattle are the main natural reservoir of EHEC (Nataro & Kaper, 1998, Yoon & Hovde, 2008; Lim et al., 2010). EHEC has several serotypes; however, the serotype O157:H7 has been associated with several outbreaks in developed countries (Donnenberg & Whittam, 2001; Kaper, 1998; Lim et al., 2010). This serotype is known to cause acute gastroenteritis, hemorrhagic

colitis (HC) and the often fatal hemolytic uremic syndrome (HUS) (Donnenberg & Whittam, 2001; Frankel et al., 1998; Kaper et al., 2004; Lim et al., 2010). Nowadays, EHEC is recognized as a global health problem mainly in developed countries (Kaper, 1998; Kaper et al., 2004; Nataro & Kaper, 1998; Lim et al., 2010).

The main virulence factor in EHEC O157:H7 is the Shiga toxins (Donnenberg & Whittam, 2001; Kaper et al., 2004; Nataro & Kaper, 1998; Lim et al., 2010) which is involved in the gastrointestinal damage resulting in bloody diarrhea, hemorrhagic colitis, necrosis, and intestinal perforation (Kaper et al., 2004). The Shiga toxins are cytotoxins encoded by a bacteriophage that is in the chromosome bacterial (Nataro & Kaper, 1998; Lim et al., 2010). There are two different proteins Stx1 and Stx2, which have similar biological activities (Hussein, 2007; Kaper, 1998; Kaper et al., 2004; Nataro & Kaper, 1998). Shiga toxin has two subunits A1B5 (Donnenberg & Whittam, 2001; Kaper et al., 2004; Robins-Browne & Hartland, 2002). The five subunits B of the toxin bind to globotriaosyl ceramide (Gb3) or globotetraosyl ceramide (Gb4) while the subunit A1 can enter to the cell by endocytosis (Nataro & Kaper, 1998; Lim et al., 2010) in order to be transported by Golgi apparatus (Donnenberg & Whittam, 2001; Kaper, 1998). In the reticulum endoplasmatic, A1, which is enzymatically active, acts with 60S ribosomal subunit, disrupting the ribosome integrity; as a consequence, it affects the inhibition of proteins synthesis in the host cells causing epithelial cell death by apoptosis (Donnenberg & Whittam, 2001; Kaper et al., 2004; Nataro & Kaper, 1998; Lim et al., 2010).

After the signal transduction response caused by Shiga toxins, the pathogenesis of EHEC O157:H7 is also characterized by attaching and effacing adherence pattern to the epithelium gastrointestinal causing A/E lesions (Frankel et al., 1998; Kaper, 1998; Nataro & Kaper, 1998; Lim et al., 2010). EHEC O157:H7 has the pathogenicity island in where is

the locus of enterocyte effacement (LEE) (Kaper, 1998; Lim et al., 2010), which encodes a type III secretion system, secreted proteins, intimin, and intimin receptor (Tir) as well as EPEC (Donnenberg & Whittam, 2001; Kaper et al., 2004; Lim et al., 2010). Additionally, EHEC 0157:H7 contain a virulence plasmid of called p0157 (Kaper, 1998; Nataro & Kaper, 1998; Lim et al., 2010). The plasmid encoded other virulence factors involved in the infection cause by EHEC 0157:H7 (Kaper, 1998; Lim et al., 2010) such as a type II secretion system (etp), a secrete serine protease (espP), a hemolysin (ehxA) (Kaper, 1998), a catalase-peroxide (katP) (Donnenberg & Whittam, 2001), a putative adhesin (toxB), a zinc metalloprotease (stcE), and an *eae* conserved fragment (ecf) (Lim et al., 2010). Although EHEC O157:H7 has an *eae* conserved fragment, it does not the genes that encoded a fimbria called bundle-forming pilus (*bfp*) but has homolog genes that encoded a linphostatin (Donnenberg & Whittam, 2001).

2.5. Enteroaggregative *Escherichia coli* (EAEC)

Enteroaggregative *Escherichia coli* is considered an emergent pathogen since 1987, characterizing by its aggregative way to adhere to epithelial cells (Mathewson et al., 1987, Nataro & Kaper, 1998; Nataro et al., 1998; Nguyen et al., 2005). EAEC is mainly associated to acute and persistent diarrhea in children (Bhan et al., 1989; Cennimo et al., 2009) and AIDS patients (Huang et al., 2006; Law & Chart, 1998; Medina et al., 2010; Weintraub, 2007). In the other hand, EAEC constitute a second cause of diarrhea in travelers (Huang et al., 2007; Huang et al., 2008; Law & Chart, 1998; Pabs et al., 2003). The principal route of transmission is fecal oral due to contaminated food and water as a consequence of poor sanitation (Huang et al., 2004; Huang et al., 2006; Law & Chart, 1998; Scavia et al., 2008). In addition, the pathogenesis of EAEC is not well understood

but the lesions that EAEC causes are different than those provoked by the others DEC (Law & Chart, 1998; Nataro et al., 1998).

The most important factor of virulence of EAEC is the presence of a plasmid called pAA, in which is the principal virulence genes associated with the aggregative adherence pattern (AA) (Law & Chart, 1998; Regua-Mangia et al., 2009). Depending on the presence of the plasmid pAA, EAECs are classified in two groups: typical and atypical. The typical EAEC has a plasmid called pAA of 60-65 MDa while atypical EAEC lacks the plasmid (Huang et al., 2004). The plasmid encoded several virulence factors such as a heat stable toxin-1 (EAST-1), Shigella enterotoxin (ShET1), cytophatic serine protease autotransporter (Pet), aggregative fimbriae I, II, and III (Nataro & Kaper, 1998; Nataro et al., 1998), a cryptic ORF (shf), and other outer membrane adhesins of 18 and 30 kDa (Weintrud, 2007). In the other hand, the plasmid has a transcriptional activator gene (*AggR*) to expression AAF-1 (Nataro et al., 1994), anti-aggregation protein transporter gene (dispersin) (Huang et al., 2004; Sheikh et al., 2002). EAEC has a pathogenicity island that contains enterotoxin (ShET1), mucinase (Pic) genes, and a *Yersinia* high-pathogenicity island that contain *yersiniabactin siderophore* gene (Huang et al., 2004; Weintraub, 2007).

Although the pathogenesis of EAEC is less understood, many studies suggest a model of its pathogenesis (Huang et al., 2006; Weintraub, 2007) which consists in three phases (Nataro et al., 1998; Huang et al., 2006): adherence to the intestinal mucosa, production of enterotoxins and cytotoxins, and inflammation (Harrington, et al., 2006). The first phase is the adherence to the intestinal mucosa by aggregative adherence fimbria (AAF) or other adherence factors (Huang et al., 2006; Nataro et al., 1998). Proteins such as AAF-1, AAF-2, and AAF-3 encoded by *aggA*, *aafA*, and *agg3* genes respectively, facilitate the adherence (Huang et al., 2006). AAF-1 allows the aggregation and haemagglutination of

the erythrocyte, AAF-II is implicated in intestinal mucosal adherence, and AAf-3 is an adhesin (Huang et al., 2004). The other adherence factors are three membrane-associated proteins (MAPs) that allow the adhesion and the haemagglutination (Huang et al., 2004; Huang et al., 2006). In the other hand, AggR control the expression of dispersin (aap), protein responsible for spreading of EAEC crossways the intestinal mucosa to allow an efficient adherence and aggregation (Huang et al., 2006). In the second phase, both bacteria and the host cells produce a mucus layer that production of mucus depends on two chromosomal gene *Fis* and *yafK* that encoded a DNA binding protein and a secreted protein respectively (Huang et al., 2006). The mucus production produces a biofilm which is deposited in the surface of enterocyte as a result it promote a persistent colonization as a consequence there are an extended diarrhea and a bad absorption of nutrients (Huang et al., 2006; Nataro et al., 1998). Finally, EAEC produce cytotoxins and enterotoxins such as Pet, EAST1, ShET1 that produce intestinal toxicity and an inflammatory response resulting in a secretory diarrhea and mucosal damage (Huang et al., 2006; Huang et al., 2007; Law & Chart, 1998; Nataro et al., 1998).

2.6. Diffusely adherent *Escherichia coli* (DAEC)

According to the patterns of adherence to the host cells are recognized tree different patterns: localized, aggregative, and diffuse (Scaletsky et al., 1984). Localized adherence is associated to EPEC, which adhere to one or a few localize sites of cells (Law & Chart, 1998; Magalhães et al., 1992; Scaletsky et al., 1984) while aggregative adhesion is characterized by an adherence only to the surface of cultured epithelial cells (Scaletsky et al., 1984). Finally, diffuse adhesion in where the microorganism adheres over the entire surface of the cells is related to diffusely adherent *Escherichia coli* (Law & Chart, 1998; Scaletsky et al., 1984).

Diffusely adherent *Escherichia coli* is the sixth category identified as a supposed cause of persistent diarrhea (Gunzburg et al., 1993; Jallat et al., 1993; Magalhães et al., 1992; Nataro & Kaper, 1998). DAEC is characterized by its diffuse pattern of adherence on Hep2 cell culture (Kaper et al., 2004; Scaletsky et al., 1984); however, its pathogenicity and epidemiology are less studied and understood (Nataro & Kaper et al., 1998). DAEC has been related to episodes of diarrhea in children less than 12 months of age (Scaletsky et al., 2002; Spano et al., 2008) although there are some studies in which DAEC was not associated with disease (Echeverria et al., 1992b; Gomes et al., 1989; Tacket et al., 1990).

DAEC is classified in two classes: the first class is those that has a fimbrial adhesive sheaths (Afas) encoded by *afa* genes, and the second one are those that express adhesin involved in the adherence pattern (AIDA-1) which is the mainly responsible of the diarrhea in children (Law, 1994; Servin, 2005). DAEC strains produce fimbrial adhesin F1845 (Kaper et al., 2004; Servin, 2005) encoded by *daaA, daaB, daaC, daaD,* and *daaE* genes (Servin, 2005; Torres et al., 2005). The fimbrial adhesin F1845 is a member of the Dr Family of adhesins that mediates the diffuse adherence pattern to the epithelial cells (Kaper et al., 2004; Servin, 2005). One subunit, DaaE, of F1845 can bind to decay accelerating factor (DAF) (Torres et al., 2005), inducing many cellular responses such as elongation, nucleation of microvilli, formation of membrane projections, rearrangements of actin, and causing inflammation (Bétis et al., 2003; Servin, 2005). In the other hand, AIDA-1 is the adhesin, a protein encoded by *aidA* gene located in a plasmid, is involved in the diffuse adherence (Benz & Schmidt, 1989; Torres et al., 2005). The protein AIDA-1 immature is an autotransporter that is glycosylated with heptoses by the autotransporter adhesion heptosyltransferase (Aah) which allow the maturation of AIDA-1 in the host cells (Torres

et al., 2005). Additionally, DAEC probably contains a homolog of the LEE locus, which can contribute to the potential pathogenesis of DAEC (Beinke et al., 1998).

3. INFECTIOUS DIARRHEA IN ECUADOR

Infectious diarrhea is one of the main causes of death in Ecuador (PAHO 2009; WHO, 2008). In 2005, infectious diarrhea was a second cause of infantile death in Ecuador. In 2008 in this country, the number of deaths due to infectious diseases were 7,022 of which 388 were caused by infectious diarrhea in children under 5 years old (Black et al., 2010), which is 6 % of all fatalities and represents the sixth cause of death in Ecuador (WHO, 2008). In Ecuador and other developing countries, infectious diarrhea is associated with poor environmental conditions (MSP, 2011), and the environmental changes can influence in the patter of disease transmission (Eisenberg et al., 2006). The inappropriate water and sanitation services increases the incidence of infectious diarrhea (MSP, 2011), also high levels of *Escherichia coli* are found in water during the dry season (Bueno, 2006; Levy et al.,2008; Levy et al., 2009) as well as in food in remote communities (Trostle et al., 2008). The quality of water in remote communities depends on factors such as rainfall, water source, and type of storage (Levy et al., 2009). The routes of transmission are related to the social and geographic in these remote communities (Bates et al., 2007; Trostle et al., 2008) which may influence also the spread of strains with antibiotic resistance such as *E. coli* (Segovia, 2008).

The main etiologic agents associated with diarrhea are viruses and bacteria in Ecuador (Endara et al., 2007; Hasing et al., 2009; Vieira et al., 2007). The principal virus associated with acute diarrhea worldwide is rotavirus (Brüssow et al., 1990b; Susuki, 1985). In 2005, a high prevalence of infection of rotavirus G9 was reported in two regions in Ecuador: remote communities in northwestern Ecuador, and in an urban Hospital (Endara, 2006;

Endara et al., 2007). However, this patters change progressively over time, G9 was replaced by G1 and G2 genotypes in the same remote areas and an urban Hospital during 2006 to 2008 (Hasing et al., 2009).

In the order hand, the main bacteria associated with diarrhea are the *Escherichia coli* in Ecuador (Brüssow et al., 1992; Schreckinger, 2008; Vieira et al., 2007). The main route of transmission is contaminated water (Bueno, 2006) and food (Trostle et al., 2008). There are a few epidemiological studies of each pathotypes of *Escherichia coli* in Ecuador (Vieira et al., 2007; Schreckinger, 2008). In 1990a and 1992, Brüssow et al. showed the presence of ETEC in Ecuadorian children using a sero-epidemiological analysis. A previous report (Vieira et al., 2007) found that EIEC was the most prevalent pathotype in 22 communities in northwestern Ecuador between 2003 and 2005. These findings contradicted those from previous studies indicating that ETEC is the most prevalent pathotype in developing countries (Okeke, 2009; Qadri et al, 2005; WHO, 2010**)**. In addition, *E. coli* Shigellae was found in greater numbers in younger group that was EIEC associated with older groups (Vieira et al., 2007). In the other hand, EPEC was found in low levels in young children in remote villages in north western Ecuador (Schreckinger, 2008).

Both reports, Vieira et al., 2007 and Schreckinger, 2008 showed the occurrence of EIEC and EPEC in asymptomatic group which constitute the reservoir of these pathotypes. In this study, we estimate the prevalence of the ETEC, EPEC, EIEC, and *E. coli* Shigellae pathotypes in 16 of the original 22 remote communities over a longer time period (2003 to 2010).

4. *ECOLOGÍA DESARROLLO SALUD Y SOCIEDAD* (EcoDess)

The project *Ecología Desarrollo Salud y Sociedad* (EcoDess) was established by Mauricio Espinel and Joseph Eisenberg in 2003. Financial support for this project was granted by the National Institute of Allergy and Infectious Diseases in association with the University of California in Berkeley represented by Joseph Eisenberg and originally Mauricio Espinel was the co-director of the project in Ecuador.

In 1996, a new highway was built linking cities of the Ecuadorian highlands to the northern coast. The new road connected Borbón to remote villages in the Canton Eloy Alfaro, Esmeraldas (Bates et al., 2001). Additionally, the new paved road increased human activity such as: logging, deforestation, poultry farming, and human migration into and out of the region (Eisenberg et al., 2006). In the other hand, the villages lacked a good sanitation systems and basic services. The original project identified infectious diarrhea as the main cause associated with children death in the area (Bueno, 2006). A case-control 15 days study was design to study the impact of remoteness on diarrheal disease and the spread of antibiotic resistance in humans. In 2003-2008, 21remote villages were tested in a case-control study. In 2005, Borbón was included and in 2009, 9 villages were added. Nowadays, 32 remote villages are part of the case control study. The villages were chosen randomly and to assure the representatively sample size was designed taking in account villages size, density, and geographical location relative to Borbón. In each community, all households were recruited; however, a random sample of 400 households was chosen in Borbón. During the field visits, fecal samples were collected from both cases and controls; additional, health workers give services and nutrition consulting. Also, a complete census of all study villages has been carried out each year since began the project (EcoDess, 2011).

5. REFERENCES

1. Albert, M., Faruque, A., Faruque, S., Sack, R., & Mahalanabis, D. (1999). Case-control study of enteropathogens associated with childhood diarrhea in Dhaka, Bangladesh. *J. Clin. Microbiol*. 37 (11): 3458-3464.

2. Alcoba-Flórez, J., Pérez-Roth, E., González-Linares, S., & Méndez-Álvarez, S. (2005). Outbreak of *Shigella sonnei* in a rural hotel in La Gomera Canary Islands, Spain. *Int. Microbiol*. 8 (2): 133-136.

3. Bates, S., Trostle, J., Cevallos, W., Hubbard, A., & Eisenberg, J. (2007). Relating diarrheal disease to social networks and the geographic configuration of communities in rural Ecuador. *Am. J. Epidemiol*. 166 (9): 1088–1095.

4. Beinke, C., Laarman, S., Wachter, C., Karch, H., Greune, L., & Schmidt A. (1998). Diffusely adhering *Escherichia coli* strains induce attaching and effacing phenotypes and secrete homologs of Esp proteins. *Infect. Immun*. 66 (2): 528-539.

5. Benz, I., & Schmidt, A. (1989). Cloning and expression of an adhesin (AIDA-I) involved in diffuse adherence of enteropathogenic *Escherichia coli*. *Infect. Immun*. 57 (5): 1506-1511.

6. Bernardini, M., Mounier, J., Hauteville, H., Coquis-Rondon, M., & Sansonetti, R. (1989). Identification of *ics*A, a plasmid locus of *Shigella flexneri* that governs bacterial intra- and intercellular spread through interaction with F-actin. *Proc. Natl. Acad. Sci*. 86: 3867-3871.

7. Bétis, F., Brest, P., Hofman, V., Guignot, J., Bernet-Camard, M. F., Rossi, B., Servin, A., & Hofman P. (2003). The Afa /Dr adhesins of diffusely adhering *Escherichia coli* stimulate interleukin-8 secretion, activate mitogen-activated protein kinases, and promote polymorphonuclear transepithelial migration in T84 polarized epithelial cells. *Infect. Immun*. 71 (3): 1068-1074.

8. Beutin, L., Gleier, K., Kontny, I., Echeverria, P., & Scheutz, F. (1997). Origin and characteristics of enteroinvasive strains of *Escherichia coli* (EIEC) isolated in Germany. *Epidemiol. Infect*. 118 (3): 199-205.

9. Bhan, M., Raj, P., Levine, M., Kaper, J., Bhandari, N., Srivastava, R., Kumar, R., & Sazawal, S. (1989). Enteroaggregative *Escherichia coli* associated with persistent diarrhea in a cohort of rural children in India. *J. Infect. Dis*. 159 (6): 1061-1064.

10. Black, R., Cousens, S., Johnson, H., Lawn, J., Rudan, I., Bassni, D., Jha, P., Campbell, H., Fisher Walker, C., Cibulskis, R., Eisele, T., Liu, L., & Mathers, C. (2010). Global, regional, national causes of child mortality in 2008: a systematic analysis. *Lancet*. 375 (9730): 1969-1987.

11. Brüssow, H., Sidoti, J., Link, H., Hoang, Y., Barclay, D., Dirren, H., & Freire W. (1990a). Age-specific prevalence of antibody to enterotoxigenic *Escherichia coli* in Ecuadorian and German children. *J. Infect. Dis*. 162 (4): 974-977.

12. Brüssow, H., Sidoti, J., Barclay, D., Sotek, J., Dirren, H., & Freire, W. (1990b). Prevalence and serotype specificity of Rotavirus antibodies in different age groups of Ecuadorian infants. *J. Infect. Dis*. 162: 615-620.

13. Brüssow, H., Rahim, H., & Freire, W. (1992). Epidemiological analysis of serologically determined Rotavirus and enterotoxigenic *Escherichia coli* infections in Ecuadorian children. *J. Clin. Microbiol*. 30 (6): 1585-1587.

14. Bueno, P. (2006). Análisis microbiológico del agua de la parroquia Borbón, cantón Eloy Alfaro y su asociación con la enfermedad diarreica. Tesis de Biotecnología. Universidad San Francisco de Quito. Quito-Ecuador.

15. Cabilio, B. (2000). Enterotoxigenic *Escherichia coli*-An overview. *Mem. Inst. Oswaldo Cruz*. 95 (1): 95-97.

16. Cennimo, D., Abbas, A., Huang, D., & Chiang, T. (2009). The prevalence and virulence characteristics of enteroaggregative *Escherichia coli* at an urgent care clinic in the USA: a case-control study. *J. Med. Microbiol*. 58: 403-407.

17. Chen, H. D., & Frankel, G. (2005). Enteropathogenic *Escherichia coli*: unravelling pathogenesis. *FEMS Microbiol. Rev*. 29 (1): 83-98.

18. Daniels, N., Neimann, J., Karpati, A., Parashar, U., Greene, K., Wells, J., Srivastava, A., Tauxe, R., Mintz, E., & Quick, R. (2000). Traveler´s diarrhea at sea: three outbreaks of waterborne enterotoxigenic *Escherichia coli* on cruise ships. *J. Infect. Dis*. 181 (4): 1491-1495.

19. Dedeić-Ljubović, A., Hukić, M., Bekić, D., & Zvizdić, A. (2009). Frequency and distribution of diarrhoeanogenic *Escherichia coli* strains isolated from pediatric patients with diarrhoea in Bosnia and Herzegovina. *Bosnian J. Basic Med. Sci*. 9 (2): 148-155.

20. Donnenberg, M., & Kaper, J. (1992). Enteropathogenic *Escherichia coli*. *Infect. Immun*. 60 (10): 3953-3961.

21. Donnenberg, M., & Whittam, T. (2001). Pathogenesis and evolution of virulence in enterophatogenic and enterohemorrhagic *Escherichia coli*. *J. Clin. Invest.* 107 (5): 539-548.

22. Echeverria, P., Sethabutr, O., Serichantalerg, O., Lexomboon, U., & Tamura, K. (1992a). *Shigella* and enteroinvasive *Escherichia coli* infections in households of children with dysentery in Bangkok. *J. Infect. Dis*. 165 (1): 144-147.

23. Echeverria, P., Serichantalerg, O., Changchawalit, S., Baudry, B., Levine, M., Orskov, F., & Orskov, I. (1992b). Tissue culture-adherent *Escherichia coli* in infantile diarrhea. *J. Infect. Dis*. 165 (1): 141-143.

24. *Ecología Desarrollo Salud y Sociedad (*EcoDess). (2011). Available at: http://www.sph.umich.edu/scr/ecodess/home.php. Accessed August 18, 2011.

25. Eisenberg, J., Cevallos, W., Ponce, K., Levy, K., Bates, S., Scott, J., Hubbard, A., Vieira N., Endara, P., Espinel, M., Trueba, G., Riley, L., & Trostle, J. (2006). Environmental change and infectious disease: How new roads affect the transmission of diarrheal pathogens in rural Ecuador. *Proc. Natl. Acad. Sci*. 103 (51): 19460-19465.

26. Endara, P. (2006). High prevalence of P(8)G9 Rotaviruses in remote communities of Ecuadorian coast. Tesis de Maestría de Microbiología. Universidad San Francisco de Quito. Quito-Ecuador.

27. Endara, P., Trueba, G., Solberg, O., Bates, S., Ponce, K., Cevallos, W., Matthijnssens, J., & Eisenberg, J. (2007). Symptomatic and subclinical infection with Rotavirus P[8]G9, rural Ecuador. *Emerg. Infect. Dis*. 13 (4): 574-580.

28. Escobar-Páramo, P., Giudicelli, C., Parsot, C., & Denamur, E. (2003). The evolutionary history of *Shigella* and enteroinvasive *Escherichia coli* revised. *J. Mol. Evol*. 57: 140-148.

29. Faundez, G., Figueroa, G., Troncoso, M., & Cabello, F. (1988). Characterization of enteroinvasive *Escherichia coli* strains isolated from children with diarrhea in Chile. *J. Clin. Microbiol*. 26 (5): 928-932.

30. Frankel, G., Phillips, A., Rosenshine, I., Dougan, G., Kaper, J., & Knutton, S. (1998). Enteropathogenic and enterohaemorrhagic *Escherichia coli*: more subversive elements. *Mol. Microbiol*. 30 (5): 911-921.

31. Frankel, G., & Phillips, A. (2008). Attaching effacing *Escherichia coli* and paradigms of Tir-triggered actin polymerization: getting off the pedestal. *Cell Microbiol*. 10 (3): 549-556.

32. Gomes, T., Blake, P., & Trabulsi, L. (1989). Prevalence of *Escherichia coli* strains with localized, diffuse, and aggregative adherence to HeLa cells in infants with diarrhea and matched controls. *J. Clin. Microbiol*. 27 (2): 266-269.

33. Gómez-Duarte, O., Arzuza, O., Urbina, D., Bai, J., Guerra, J., Montes, O., Puello, M., Mendoza, K., & Castro, G. (2010). Detection of *Escherichia coli* enteropathogens by multiplex polimerase chain reaction from children's diarrheal stools in two Caribbean-Colombian cities. *Foodborne Pathog Dis.* 7 (2):199-20.

34. Gordillo, M., Reeve, G., Pappas, J., Mathewson, J., DuPont, H., & Murray, B. (1992). Molecular characterization of strains of enteroinvasive *Escherichia coli*

O143, including isolates from a large outbreak in Houston, Texas. *J. Clin. Microbiol*. 30 (4): 889-893.

35. Guerrant, R., Van Gilder, T., Steiner, T., Thielman, N., Slutsker, L., Tauxe, R., Hennessy, T., Griffin, P., DuPont, H., Brandley, R.., Tarr, P., Neil, M., Nachamkin, I., Reller, L., Osterholm, M., Bennish, M., & Pickring, L. (2001). Practice guidelines for the management of infectious diarrhea. *Clin. Infect. Dis*. 32 (3): 331-351.

36. Gunzburg, S., Chang, B., Elliot, S., Burke, V., & Gracey, M. (1993). Diffuse and enteroaggregative patterns of adherence of enteric *Escherichia coli* isolated from aboriginal children from Kimberley region of Western Australia. *J. Infect. Dis*. 167 (3): 755-758.

37. Gupta, S., Keck, J., Ram, P., Crump, J., Miller, M., & Mintz, E. (2008). Part III. Analysis of data gaps pertaining to enterotoxigenic *Escherichia coli* infections in low and medium human development index countries, 1984-2005. *Epidemiol. Infect*. 136 (6): 721-738.

38. Harrington, S., Dudley, E., & Nataro, J. (2006). Pathogenesis of enteroaggregative *Escherichia coli* infection. *FEMS Microbiol. Lett*. 254: 12-18.

39. Harris, J., Mariano, J., Wells J., Panye, B., Donnell, H. & Cohen, M. (1985). Person to person transmission in an outbreak of enteroinvasive *Escherichia coli*. *Am. J. Epidemiol*. 122 (2): 245-252.

40. Hasing, M., Trueba, G., Baquero, M., Ponce, K., Cevallos, W., Solberg, O., & Eisenberg, J. (2009). Rapid changes in Rotaviral genotypes in Ecuador. *J. Med. Virol*. 81: 2109–2113.

41. Herrera, A., Espinosa, B., Nuñez, G., Espinoza, N., Maves, R., & Martin, G. (2010). The effect of preparation of cebiche on the survival of enterotoxigenic *Escherichia coli*, *Aeromonas hydrophila*, and *Vibrio parahaemolyticus*. *J. Travel Med*. 17 (6): 395-399.

42. Hernandes, R., Elias, W., Vieira, M., & Gomes, T. (2009). An overview of atypical enterophatogenic *Escherichia coli*. *FEMS Microbiol Lett*. 297: 137-149.

43. Huang, D., Okhuysen, P., Jiang, Z., & DuPont, H. (2004). Enteroaggregative *Escherichia coli*: an emerging pathogen. *Am. J. Gastroenterol*. 99: 383-389.

44. Huang, D., Mohanty, A., DuPont, H., Okhuysen, P., & Chiang, T. (2006). A review of an emerging enteric pathogen: enteroaggregative *Escherichia coli*. *J. Med. Microbiol*. 55: 1303-1311.

45. Huang, D., Mohamed, J., Nataro, J., DuPont, H., Jiang, Z. D., & Okhuysen, P. (2007). Virulence characteristics and the molecular epidemiology of enteroaggregative *Escherichia coli* isolates from travelers to developing countries. *J. Med. Microbiol*. 56: 1386-1392.

46. Huang, D., Brown, E., DuPont, H., Cerf, J., Carlin, L., Flores, J., Belking-Gerson, J., Nataro, J., & Okhuysen, P. (2008). Seroprevalence of the enteroaggregative *Escherichia coli* virulence factor dispersin among USA travelers to Cuernavaca, Mexico: a pilot study. *J. Med. Microbiol*. 57: 476-479.

47. Huerta, M., Grotto, I., Gdalevich, M., Mimouni, D., Gavrieli, B., Yavzori, M., Cohen, D., & Shpilberg, O. (2000). A waterborne outbreak of gastroenteritis in the Golan Height due to enterotoxigenic *Escherichia coli*. *Infection*. 28 (5): 267-271.

48. Hunter, P. (2003). Drinking water and diarrhoeal disease due to *Escherichia coli*. *J. Water and Health*. 01.2: 65-72.

49. Hussein, H. (2007). Prevalence and pathogenicity of Shiga toxin-producing *Escherichia coli* in beef cattle and their products. *J. Anim. Sci*. 85: E63-E72.

50. Ishii, S., & Sadowsky, M. (2008). *Escherichia coli* in the environment: implications for water quality and human health. *Microbes Environ*. 23 (2): 101-108.

51. Jain, S., Chen, L., Dechet, A., Hertz, A., Brus, D., Hanley, K., Wilson, B., Frank, J., Greene, K., Parsons, M., Bopp, C., Todd, R., Hoekstra, M., Mintz, E., & Ram, P. (2008). An outbreak of enterotoxigenic *Escherichia coli* associated with sushi restaurant in Nevada, 2004. *Clin. Infect. Dis*. 47 (1): 1-7.

52. Jallat, C., Livrelli, V., Darfeuille-Michaud, A., Rich, C., & Joly, B. (1993). *Escherichia coli* strains involved in diarrhea in France: high prevalence and heterogeneity of diffusely adhering strains. *J. Clin. Microbiol*. 31 (8): 2031-2037.

53. Jennison, A., & Verma, N., (2004). *Shigella flexneri* infection: pathogenesis and vaccine development. *FEMS Microbiol. Rev*. 28: 43-58.

54. Kaper, J. (1998). Enterohemorrhagic *Escherichia coli*. *Curr. Opin. Microbiol*. 1: 103-108.

55. Kaper, J., Nataro, J., & Mobley, H. (2004). Pathogenic *Escherichia coli*. *Nature Rev*. 2: 123-140.

56. Khalil, K., Khan, S., Mazhar, K., Kaijser, B., & Lindblom, G. (1998). Occurrence and susceptibility to antibiotics of *Shigella* species in stools of hospitalized children with bloody diarrhea in Pakistan. *Am. J. Trop. Med. Hyg*. 58 (6): 800-803.

57. Koletzko, S., & Osterrieder, S. (2009). Acute infectious diarrhea in children. *Dtsch Arztebl Int*. 106 (33): 539-548.

58. Kuhnert, P., Boerlin, P., & Frey, J. (2000). Target genes for virulence assessment of *Escherichia coli* isolates from water, food and the environment. *FEMS Microbiol. Rev*. 24: 107-117.

59. Kumar, H., Parvathi, A., Karunasagar, A., & Karunasagar, I. (2005). Prevalence and antibiotic resistance of *Escherichia coli* in tropical seafood. *World J. Microbiol. Biotechnol.* 21: 619-623.

60. Lan, R., Lumb, B., Ryan, D., & Reeves, P. (2001). Molecular evolution of large virulence plasmid in *Shigella* clones and enteroinvasive *Escherichia coli*. *Infect. Immun*. 69 (10): 6303-6309.

61. Law, D. (1994). Adhesion and its role in the virulence of enteropathogenic *Escherichia coli*. *Clin. Microbiol. Rev*. 7 (2): 152-173.

62. Law, D., & Chart, H. (1998). Enteroaggregative *Escherichia coli*. *J. Appl. Microbiol.* 84: 685-697.

63. Levine, M. M. (1987). *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic and enteroadherent. *J. Infect. Dis.* 155 (3): 377-379.

64. Levy, K., Nelson, K., Hubbard, A., & Eisenberg, J. (2008). Following the water: a controlled study of drinking water storage in northern coastal Ecuador. *Environ. Health Perspectives*. 16 (11): 1533-1540.

65. Levy, K., Hubbard, A., Nelson, K., & Eisenberg, J. (2009). Drivers of water quality variability in northern coastal Ecuador. *Environ. Sci. Technol*. 43 (6): 1788–1797.

66. Lim, Y., Yoon, J., & Hovde, C. (2010). A Brief overview of *Escherichia coli* O157:H7 and its plasmid O157. *J. Microbiol. Biotechnol*. 20 (1): 1-10.

67. Lothigius, Å., Janzon, A., Begum, Y., Sjöling, Å., Qadri, F., Svennerholm, A. M., & Bölin, I. (2008). Enterotoxigenic *Escherichia coli* is detectable in water samples from an endemic area by real-time PCR. *J. Appl. Microbiol.* 104: 1128-1136.

68. Lothigius, Å., Sjöling, Å., Svennerholm, A. M., & Bölin, I. (2010). Survival and gene expression of enterotoxigenic *Escherichia coli* during long-term incubation in sea water and freshwater. *J. Appl. Microbiol.* 108: 1441-1449.

69. MacDonald, K., Eidson, M., Strohmeyer, C., Levy, M., Wells, J., Puhr, N., Wachsmuth, K., Hargrett, N., & Cohen, M. (1985). A multistate outbreak of gastrointestinal illness caused by enterotoxigenic *Escherichia coli* in imported semisoft cheese. *J. Infect. Dis.*151 (4): 716-720.

70. Magalhães, M., Amorim, R., Takeda, Y., Tsukamoto, T., Antas, M., & Tateno, S. (1992). Localized, diffuse, and aggregative-adhering *Escherichia coli* from infants with acute diarrhea and matched-controls. *Mem. Inst. Oswaldo Cruz*. 87 (1): 93-97.

71. Mathewson, J., Oberhelman, R., DuPont, H., De La Cabada, J., & Vasquez, E. (1987). Enteroadherent *Escherichia coli* as a cause of diarrhea among children in Mexico. *J. Clin. Microbiol*. 24 (10): 1917-1919.

72. Medina, A., Rivera, F., Romero, L., Kolevic, L., Castillo, M., Verne, E., Hernandez, R., Mayor, Y., Barletta, F., Mercado, E., & Ochoa, T. (2010). Diarrheagenic *Escherichia coli* in human immunodeficiency virus (HIV) pediatric patients in Lima Peru. *Am. J. Trop. Med. Hig*. 83 (1): 158-163.

73. *Ministerio de Salud Pública del Ecuador* (MPS), (2011). *Gestión Integral de Desechos Infecciosos*. Available at: http://www.msp.gov.ec/. [Accessed August 18, 2011].

74. Nataro, J., Yikang, D., Yingkang, D., & Walker, K. (1994). AggR, a transcriptional activator of aggregative adherence fimbria I expression in enteroaggregative *Escherichia coli*. *J. Bacteriol.* 176 (15): 4691-4699.

75. Nataro, J. P., & Kaper, J. B. (1998). Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev*. 11 (1):142-201.

76. Nataro, J., Steiner, T., & Guerrant, R. (1998). Enteroaggregative *Escherichia coli*. *Emerg. Infect. Dis*. 4 (2) 251-261.

77. Nguyen, T., Van, P., Huy, C., Nguyen, K., & Weintraub, A. (2005). Detection characterization of diarrheagenic *Escherichia coli* from young children in Hanoi, Vietnam. *J. Clin. Microbiol*. 43 (2): 755-760.

78. Niyogi, S. (2005). Shigellosis. *J. Microbiol.* 43 (2): 133-143.

79. Nougayrède, J., Fernandes, P., & Donnenberg, M. (2003). Adhesion of enteropathogenic *Escherichia coli* to host cells. *Cell. Microbiol.* 5 (6): 359-372.

80. Nwachuku, N., & Gerba, C. (2008). Occurrence and persistence of *Escherichia coli* 0157:H7 in water. *Rev. Environ. Sci. Biotechnol.* 7 (3):267-273.

81. Ochoa, T., Barletta, F., Contreras, C., & Mercado, E. (2008). New insights into the epidemiology of enteropathogenic *Escherichia coli* infection. *Trans. R. Soc. Trop. Med. Hyg*. 102 (9): 852-856.

82. Ogawa, M., & Sasakawa, C. (2006). *Shigella* and autophagy. *Landes Bioscience.* 2 (3): 171-174.

83. Ogawa, M., Handa, Y., Ashida, H., Susuki, M., & Sasakawa, C. (2008). The versatility of *Shigella* effectors. *Nature Rev. Microbiol*. 6: 11-16.

84. Okeke, I., Lamikanra, A. Steinrück, H., & Kaper, J. (2000). Characterization of *Escherichia coli* strains from cases of childhood diarrhea in provincial southwestern Nigeria. *J. Clin. Microbiol*. 38 (1): 7-12.

85. Okeke, I. (2009). Diarrheagenic *Escherichia coli* in Sub-Saharan Africa: status, uncertainties and necessities. *J. Infect. Dev. Ctries*. 3 (11): 817-842.

86. Orsi, R., Stoppe, N., Sato, M., Prado, P., & Ottoboni, L. (2008). Phylogenetic group distribution among *Escherichia coli* isolated from rivers in São Paulo State, Brazil. *World J. Microbiol. Biotechnol*. 24: 1573-1577.

87. Pabs, W., Altwegg, M., Kind, C., Mirjanic, S., Hardegger, D., & Nadal, D. (2003). Prevalence of enteroaggregative *Escherichia coli* among children with and without diarrhea in Switzerland. *J. Clin. Microbiol.* 41 (6): 2289-2293.

88. Pan American Health Organization (PAHO), (2009). Mortality database browser. Total deaths by country, year, sex, age group and causes of deaths. Chapter Ecuador. Available at: http://www.paho.org. [Accessed August 18, 2011].

89. Paniagua, M., Espinoza, F., Ringman, M., Reizenstein, E., Svennerholm, A. M., & Hallander, H. (1997). Analysis of incidence of infection with enterotoxigenic *Escherichia coli* in a prospective cohort study of infant diarrhea in Nicaragua. *J. Clin. Microbiol.* 35 (6): 1404-1410.

90. Parsot, C. (2005). *Shigella* spp. and enteroinvasive *Escherichia coli* pathogenicity factors. *FEMS Microbiol Lett*. 252: 11-18.

91. Philpott, D., Edgeworth, J., & Sansoneti, P. (2000). The pathogenesis of *Shigella flexneri* infection lessons from *in vitro* and *in vivo* studies. *Phil. Trans. R. Soc. Lond.* 355: 575-586.

92. Pupo, G., Lan, R., & Reeves, P. (2000). Multiples independent origins of *Shigella* clones of *Escherichia coli* and convergent evolution of many of their characteristics. *Proc. Natl. Acad. Sci*. 97 (19): 10567-10572.

93. Qadri, F., Kumar, S., Farunque, A., Fuchs, G., Albert, M., Sack, B., & Svennerholm, A. M. (2000). Prevalence of toxin types and colonization factors in enterotoxigenic *Escherichia coli* isolated during a 2-year period from diarrheal patients in Bangladesh. *J. Clin. Microbiol*. 38 (1): 27-31.

94. Qadri, F., Svennerholm, A. M., Faruque, A., & Sack, R. (2005). Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features treatment, and prevention. *Clin. Microbiol. Rev*. 18 (3). 465-483.

95. Rahman, I., Shahamat, M., Kirchman, P., Russek-Cohen, E., & Colwell, R. (1994). Methionine uptake and cytopathogenicity of viable but noculturable *Shigella dysenteriae* type 1. *Appl. Environ. Microbiol.* 60 (10): 3573-3578.

96. Regua-Mangia, A., Gomes, T., Vieira, M., Irino, K., & Teixeira, L. (2009). Molecular typing and virulence of enteroaggregative *Escherichia coli* strains isolates from children with and without diarrhea in Rio de Janeiro city, Brazil. *J. Med. Microbiol.* 58: 414-422.

97. Robins-Browne, R., & Hartland, E. (2002). *Escherichia coli* as a cause of diarrhea. *J. Gastroenterol Hepatol*. 17: 467-475.

98. Roszak, D. & Colwell, R. (1987). Survival strategies of bacteria in the natural environment. *Microbiol. Rev*. 51 (3): 365-379.

99. Rudolph, J., & Cohen, M. (1999). New causes and treatments for infectious diarrhea in children. *Curr. Gastroenterol. Rep*. 1: 238-244

100. Sanders, J., & Tribble, D. (2001). Diarrhea in the returned traveler. *Curr. Gastroenterol. Rep*. 3: 304-314.

101. Sansonetti, P. (2001). Rupture, invasion and inflammatory destruction of the intestinal barrier by *Shigella*, making sense of prokaryote-eukaryote cross-talks. *FEMS Microbiol. Rev*. 25: 3-14.

102. Sasakawa, C. (1997). Early states of *Shigella* interaction with host cells. *J Infect Chemother*. 3 (2): 63-72.

103. Sasakawa, C. (2010). A new paradigm of bacteria-gut interplay brought through the study of *Shigella*. *Proc. Jpn. Acad.*, Ser B86 (3): 229-243.

104. Scaletsky, I., Silva, L., & Trabulsi, L. (1984). Distinctive patterns of adherence of enteropathogenic *Escherichia coli* HeLa cells. *Infect. Immun*. 45 (2): 534-536.

105. Scaletsky, I., Fabbricotti, S., Carvalho, R., Nunes, C., Maranhão, H., Morais, M., & Fagundes-Neto, U. (2002). Diffuse adherent *Escherichia coli* as a cause of acute diarrhea in young children in northeast Brazil: a case-control study. *J. Clin. Microbiol*. 40 (2): 645-648.

106. Scaletsky, I., Aranda, K., Souza, T., Silva, N., & Morais, M. (2009). Evidence of pathogenic subgroups among atypical enteropathogenic *Escherichia coli* strains. *J. Clin. Microbiol*. 47 (11): 3756-3759.

107. Scavia, G., Staffolani, M., Fisichella, S., Striano, G., Colletta, S., Ferri, G., Escher, M., Minelli, F., & Caprioli, A. (2008). Enteroaggregative *Escherichia coli* associated with foodborne outbreaks of gastroenteritis. *J. Med. Microbiol*. 57: 1141-1146.

108. Segovia, R. (2008). Evidence of horizontal gene transfer of antibiotic resistance genes in communities with limitated access to antibiotics. Tesis de Maestría de Microbiología. Universidad San Francisco de Quito. Quito-Ecuador.

109. Servin, A. (2005). Pathogenesis of Afa/Dr diffusely adhering *Escherichia coli*. *Clin. Microbiol. Rev.* 18 (2): 264-292.

110. Shaheen, H., Khalil, S., Rao, M., Abu Elyazeed, R., Wierzba, T., Peruski, L., Putnam, S., Navarro, A., Morsy, B., Cravioto, A., Clemens, J., Svennerholm, A. M., & Savarino, S. (2004). Phenotypic profiles of enterotoxigenic *Escherichia coli* associated with early childhood diarrhea in rural Egypt. *J. Clin. Microbiol*. 42 (12): 5588-5595.

111. Shah, N., DuPont, H., & Ramsey, D. (2009). Global etiology of travelers´ diarrhea: systematic review from 1973 to the present. *Am. J. Trop. Med. Hyg*. 80 (4): 609-614.

112. Sheikh, J., Czeczulin, J., Harrington, S., Hicks, S., Henderson, I., Le Bouguénec, C., Gounon, P., Phillips, A., & Nataro, J. (2002). A novel dispersin protein in enteroaggregative *Escherichia coli*. *J. Clin. Invest.* 110 (9): 1329-1338.

113. Schreckinger, M. (2008). Genotipificación de cepas de *Escherichia coli* enteropatogénica aisladas en una región rural de la costa ecuatoriana mediante el uso de la electroforesis de campo pulsado. Tesis de Biotecnología. Universidad San Francisco de Quito. Quito-Ecuador.

114. Schroeder, G., & Hilbi, H. (2008). Molecular pathogenesis of *Shigella* spp.: controlling host cell signaling, invasion, and death by type III secretion. *Clin. Microbiol. Rev.* 21 (1):134-156.

115. Spano, L., Sadovsky, D., Segui, P., Saick, K., Kitagawa, S., Pereira, F., Fagundes-Neto, U., & Scaletsky, I. (2008). Age-specific prevalence of diffusely adherent *Escherichia coli* in Brazilian children with acute diarrhoea. *J. Med. Microbiol.* 57: 359-363.

116. Susuki, H., Sato, T., Kitaoka, S., Tazawa, F., Konno, T., Amano, Y., Alava-Alprecht, A., Gutiérrez-Vera, E., López-Villalta, J., Numazaki, Y., & Ishida, N. (1985). A human atypical Rotavirus found in an Ecuador infant with diarrhea. *Tohoku J. exp. Med*. 146: 481-482.

117. Tacket, C., Moseley, S., Kay, B., Losonsky, G., & Levine, M. (1990). Challenge studies in volunteers using *Escherichia coli* strains with diffuse adherence to HEp-2 cells. *J. Infect. Dis*. 162 (2). 550-552.

118. Taylor, D., Echeveria, P., Sethabutr, O., Tarangsi, C., Leksomboon, U., Blacklow, N., Rowe, B., Gross, R., & Cross, J. (1988). Clinical and microbiologic features of *Shigella* and enteroinvasive *Escherichia coli* infections detected by DNA hybridization. *J. Clin. Microbiol*. 1362-1366.

119. Taylor, C. (2008). Enterohaemorrhagic *Escherichia coli* and *Shigella dyseteriae* type 1-induced haemolytic uraemic syndrome. *Pediatr Nephrol*. 23: 1425-1431.

120. Torres, A. (2004). Current aspects of *Shigella* pathogenesis. *Rev. Latinoam. Microbiol.* 46 (3-4): 89-97.

121. Torres, A., Zhou, X., & Kaper, J. (2005). Adherence of diarrheagenic *Escherichia coli* strains to epithelial cells. *Infect. Immun*. 73 (1): 18-29.

122. Trabulsi, L., Keller, R., & Tardelli, T. (2002). Typical and atypical enteropathogenic *Escherichia coli*. *Emerg. Infect. Dis*. 8 (5): 508-513.

123. Trostle, J., Hubbard, A., Scott, J., Cevallos, W., Bates, S., & Eisenberg, J. (2008). Raising the level of analysis of food-borne outbreaks food-sharing networks in rural coastal Ecuador. *Epidemiol.* 19 (3): 384–390.

124. Whitten, N. (1965). Class kinship and power in an Ecuadorian town: the negroes of San Lorenzo. Stanford, CA: Standford University Press.

125. Vieira, N., Bates, S., Solberg, O., Ponce, K., Howsmon, R., Cevallos, W., Trueba, G., Riley, L., & Eisenberg, J. (2007). High prevalence of enteroinvasive *Escherichia coli* isolated in a remote region of northern coastal Ecuador. *Am. J. Trop. Med. Hyg*. 76 (3): 528-533.

126. Wang, J., Zhang, X., Peng, J., Yang, E., Bin, W., Yang, J., Dong, J., Sun, L., Xu, X., & Jim, Q. (2006). Genomic composition and phylogenetic analysis of *Shigella boydii* subgroup. *Science in China: Series C Life Science.* 49 (1): 46-52.

127. Weintraub, A. (2007). Enteroaggregative *Escherichia coli*: epidemiology, virulence and detection. *J. Med. Microbiol*. 56: 4-8.

128. Wolf, M. (1997). Occurrence, distribution, and association of O and H serogroups, colonization factor antigens, and toxins of enterotoxigenic *Escherichia coli*. *Clin. Microbiol. Rev*.10 (4) 569-584.

129. World Health Organization, (2008). Ecuador Health Profile. Available at: http://www.who.int/gho/countries/ecu/country_profiles/en/. [Accessed August 18, 2011].

130. World Health Organization, (2010). Diarrhea disease. Available at: http://www.who.int/topics/diarrhoea/en/. [Accessed August 15, 2010].

131. Yang, E., Bin, W., Peng, J., Zhang, X., Wang, J., Yang, J., Dong, J., Chu, Y., Zhang, J., & Jin, Q. (2005). Comparative genomics and phylogenetic analysis of *S. dysenteriae* subgroup. *Science in China: Ser. C Life Sciences*. 48 (4): 406-413.

132. Yang, J., Nie, H., Chen, L., Zhang, X., Yang, F., Xu, X., Zhu, Y., Yu, L., & Jim, Q. (2007). Revising the molecular evolutionary history of *Shigella* spp. *J. Mol. Evol*. 64 (1): 71-79.

133. Yoon, J., & Hovde, C. (2008). All blood, no stool: enterohemorrhagic *Escherichia coli* O157:H7 infection. *J. Vet. Sci.* 9 (3): 219-231.

PART II

Temporal Changes in Prevalence of *Escherichia coli* Pathotypes in Remote Communities of Ecuador

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1. ABSTRACT

Enterotoxigenic *Escherichia coli* (ETEC) is the most prevalent *Escherichia coli* (*E. coli*) pathotype associated with infectious diarrhea in developing countries. However, a previous study found that enteroinvasive *E. coli* (EIEC) was the more prevalent pathotype in 22 remote communities in the northern coastal Ecuador between 2003 and 2005. The purpose of the present study was to determine whether the prominence of this pathotype continued after 2005 and to capture prevalence trends of four diarrheagenic *E. coli* pathotypes between August 2003 and December 2010. The analysis included 4,196 fecal samples collected from16 remote communities. PCR was used to detect virulence genes of ETEC, EIEC, EPEC, and *E. coli* Shigellae. Our findings suggest that the high prevalence of EIEC between 2005 and 2006 is the result of an outbreak in the 16 communities. In all other years, ETEC was the most prevalent pathotype in the region.

2. INTRODUCTION

Infectious diarrhea is one of the most common health problems worldwide; it is the second cause of death in children living in developing countries (Guerrant et al., 2001; WHO, 2010) and it is commonly associated with the intake of either contaminated water or food (Okeke, 2009; Qadri et al., 2005; WHO, 2010). The most common bacterial agents associated with diarrhea are *Escherichia coli* (*E. coli*) pathotypes endemic to developing countries (Okeke, 2009; Qadri et al, 2005; WHO, 2010). There are at least eight pathotypes of diarrheagenic *E. coli* (PATHOTYPE): enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), entero-aggregative (EAEC), enterohemorragic (EHEC), diffusely adherent (DAEC) (Kaper et al., 2004; Levine, 1987; Nataro & Kaper, 1998), *E. coli* Shigellae (formerly known as *Shigella* spp.) (Lan et al., 2001; Sasakawa, 1997; Yang et al., 2007), and cell-detaching *E. coli* (CDEC) (Nataro & Kaper, 1998).

A previous report (Vieira et al., 2007) found that EIEC was the most prevalent pathotype in 22 communities in northwestern Ecuador between 2003 and 2005. These findings contradict those from previous studies indicating that ETEC is the most prevalent pathotype in developing countries (Okeke, 2009; Qadri et al, 2005; WHO, 2010). ETEC has been implicated in water-borne outbreaks (Brüssow et al., 1992; Daniels et al., 2000; Huerta et al., 2000), and in undercooked seafood (Herrera et al., 2010; Jain et al., 2008; Kumar et al., 2005). EIEC is transmitted by food manipulation and person-to-person contact (Harris et al., 1985), an unusual route for ETEC (Daniels et al., 2000; Hunter, 2003; MacDonald et al., 1985). Additionally EIEC and *E. coli* Shigellae are more sensitive to environmental pH fluctuations and environmental microbiota (Nwachuku & Gerba, 2008; Rosak & Cowell, 1987) and compete poorly with other bacteria (Alcoba-Flórez et al., 2005; Faundez et al., 1988). However, it has been reported that *E. coli* Shigellae can

survive for up 6 months in aquatic environments (Khalil et al., 1998; Rahman et al., 1994). In this study, we estimate the prevalence of the ETEC, EPEC, EIEC, and *E. coli* Shigellae pathotypes in16 of the original 22 remote communities over a longer time period (2003 to 2010).

3. MATERIALS AND METHODS

3.1. Study area

Serial case control studies were conducted in 16 remote communities of northern coastal Ecuador between August 2003 and December 2010. Fifteen of these communities are situated in the canton Eloy Alfaro located on one of three river systems: the Cayapas, the Onzole, and the Santiago. These river systems drain into Borbón, which is the largest community in the study and the main population center in the region. Borbón is more densely populated than the other communities and it has a mix of undeveloped infrastructure including minimal water and sanitation services. In contrast, the other communities use the river as their primary water source and have unimproved sanitation facilities as defined by the World Health Organization criteria. The region is mainly inhabited by Afro-Ecuadorians, with a smaller proportion of indigenous Chachis; recently there has been an increasing of a number of mestizos (people of mixed origin) due to in-migration into the region (Whitten, 1965). All protocols were approved by the University of Michigan institutional review board and Universidad San Francisco de Quito's bioethics committees.

3.2. Sample collection

During each 15-day case control period fecal samples were collected from both cases and controls in the community. A case of diarrhea was defined as having three or more episodes of liquid depositions in the last 24 hours while a control was someone who had not had diarrhea in the previous six days. Between 2003 and 2008, one household- and two community-controls were randomly sampled per case. In 2009, 10% of all non-cases

in the community were randomly sampled as controls. We conducted case controls in each community approximately every eight months between 2003 and 2010 with the exception of Borbón, which we began sampling from in 2005. We refer to this eight-month period as a cycle in the subsequent text.

3.3. Bacterial culture

Samples were cultured on the following media: Xilose Lisine Desoxicholate Agar (XLD), *Salmonella* and *Shigella* Agar (SS), and MacConkey Agar (MKL). *E. coli* was identified by selecting lactose fermenting colonies and testing β-glucoronidase activity using Chromocult ® Coliforms Agar (Merck, Darmsladt, Germany) (CC). Lactose negative colonies were analyzed using API ® 20 E (BioMérieux, Marcy l'Etoile, France).

3.4. PCR analysis of *Escherichia coli* pathotypes

Escherichia coli isolates were cultured in nutrient agar for 24 hours at 37 ° C. The five colonies were resuspended in 300 µl of sterile water and were then boiled for ten minutes in order to release the DNA (pooled DNA sample). Cell suspensions were centrifuged at 65.4 g for 1 minute in order to precipitate cell debris and supernatants were maintained at – 20 °C until analyzed. Finally, 1 µl of the supernatants were used as the DNA templates for PCR (Tornieporth et al., 1995).

In order to detect the presence of pathotype-specific virulence genes (*Bfp*, *LT* gene, *STa* gene, and *ipaH)* in lactose fermenting colonies, we used the PCR protocol previously described by Tornieporth et al. (1995). Lactose negative *E. coli* was also tested for the

presence of *ipaH* gene. Promega (Madison, Wisconsin, U.S.A.) Go-flexi® DNA polymerase kit was used for PCR. The cycling conditions were those described previously by Tornieporth et al. (1995). Positive controls were kindly provided by Dr. Lee W. Riley (University of California, Berkeley). Components of positive pooled samples of lactose fermenting colonies were analyzed individually.

3.5. Statistical analysis

All analyses were carried out using R. v 2.11.1. The prevalence of infection was calculated using a weighted sum of cases and controls. Cases and controls were weighted by the inverse of their sampling probabilities. Since we assumed that all cases were identified during a 15-day visit to a community, cases were given a weight of one. Controls weights reflected a random sampling of houses and communities. We used the Horvitz-Thompson theory to estimate prevalence and their 95% confidence intervals (Horvitz & Thompson, 1952). The prevalence was estimated as follows:

$$P = \frac{\sum (\text{pathogen-positive case x weight}) + \sum (\text{pathogen-positive control x weight})}{\sum \text{weight (total cases and control)}}$$

The prevalence of cases and controls was estimated as follows:

$$P = \frac{\sum (\text{pathogen-positive case x weight})}{\sum \text{weight (total cases)}}$$

$$P = \frac{\sum (\text{pathogen-positive control x weight})}{\sum \text{weight (total controls)}}$$

Prevalences for ETEC and EPEC were calculated using data from cycles 1 to 9 (August 2003 to December 2010) while those for EIEC and *E. coli* Shigellae were calculated using data from cycles 3 to 9 (November 2004 to December 2010).

The pathogenicity of each *E. coli* pathotype was estimated by the ratio of diarrhea risk in those exposed to the pathogen to risk in those unexposed, which we refer to as a risk ratio (RR). The risk ratio was estimated as follow:

$$RR = \frac{a/(a+b)}{c/(c+d)}$$

Where: a = number of weighted positive cases, b = number of weighted positive controls, c = number of weighted negative cases, and d = number of weighted negative controls

Bootstrap 95% confidence intervals of RR were obtained by resampling from the original dataset 1000 times, estimating the RR each time, and taking the 0.025 and 0.975 percentiles of the final RR distribution.

A test for downward trend in EIEC prevalence between cycles 4 and 9 was calculated using a generalized linear model with EIEC infection as an outcome, cycle as a predictor and an offset for the weights.

4. RESULTS

4.1. Prevalence and pathogenicity of *Escherichia coli* pathotypes

A total of 4,196 fecal samples (916 cases and 3280 controls) were collected from 16 remote villages in northern coastal Ecuador between August 2003 and December 2010. Pathogenic *E. coli* was found in 275 samples (130 cases and 145 controls). A few samples came from co-infected individuals: four samples were positive for EIEC and ETEC, one sample for ETEC and *E. coli* Shigellae, and one sample for EPEC and *E. coli* Shigellae.

A summary of the *E. coli* isolates obtained from 2003 to 2010 is shown in Table 1. Overall, ETEC was the most prevalent pathotype, and was found in 0.05 to 3.71 percent of the population between August 2003 and December 2010. Of the ETEC infections, ETEC-LT was the most common with a prevalence range of 0.48 to 2.94 cases per 100 persons, followed by ETEC-ST (0 – 1.48 cases per 100 persons) between November 2004 and December 2010 (Table 3)**.** The second most prevalent pathotype was EIEC (0.97 - 4.44 cases per 100 persons), followed by *E. coli* Shigellae (0 - 1.67 cases per 100 persons) between November 2004 and December 2010. Finally, EPEC was the least common pathotype (0.02 - 1.28 cases per 100 persons, Table 1).

Of the four pathotypes detected, *E. coli* Shigellae was most strongly associated with diarrhea (RR = 6.90, (95% CI: 3.76, 13.69), while EIEC was least associated with diarrhea (RR = 1.15, (95% CI: 0.61, 1.96), Table 4).

4.2. Temporal distribution of pathotypes

Prevalence of the four *E. coli* pathotypes changed over time in the 16 remote communities of northern coastal Ecuador (Table 1). In the communities around Borbón, EIEC was the most prevalent pathotype between August 2005 and March 2006(4.44 cases per 100 persons) followed by ETEC (0.76 cases per 100 persons), *E. coli* Shigellae (0.25 cases per persons), and EPEC (0.15 cases per person). Between May 2006 and December 2010, ETEC was most prevalent. Prevalence of ETEC ranged from 1.4 to 3.7 cases per 100 persons. During this time, prevalence of EIEC steadily decreased (OR: 0.80, (95 % CI: 0.69, 0.91), Figure 1). In Borbón, EIEC was the most prevalent pathogenic *E. coli* between August 2005 and March 2006 (18.5 cases per person, Table2) and unlike in the other communities, in July 2007 (3.1 cases per person). During all other cycles, ETEC was the most prevalent pathotype in Borbón.

Amongst the ETEC isolates, the most common were ETEC-LT, followed by ETEC-ST November 2004 and March 2008 (Table 3). After March 2008, ETEC carrying the ST toxin were most prevalent, with 1.48 and 0.61 cases per 100 persons in 2009 and 2010 respectively. In the same period, the prevalence of ETEC-LT was 0.71 and 0.58 cases per 100 persons, and the prevalence of ETEC-LT/ST was 0 and 0.44 cases per 100 persons (Table 3).

5. DISCUSSION

In a previous study carried out in remote communities of northwestern Ecuador from 2003 to 2005 our research group found that EIEC was the most prevalent pathotype in this region (Vieira et al., 2007). The present study carried out in the same region between 2006 and 2010, showed that ETEC was the most prevalent over the 5-year period. This finding is in agreement with previous reports indicating that ETEC is one of the main causes of diarrhea in Ecuador (Brüssow et al., 1990; Brüssow et al., 1992) and the main cause of waterborne outbreaks in this country (Brüssow et al., 1992) as well as in other developing countries (Huerta et al., 2000).Additionally, we observed that the prevalence of *E. coli* pathotypes tend to vary overtime. For instance, EIEC was the pathotype most prevalent in all 16 communities between August 2005 and March 2006.Moreover, this pathotype was the most common in Borbón between February and July 2007. In comparison, *E. coli* Shigellae was not found in any community between December 2008 and November 2009 (Figure 1).

Increases in EIEC prevalence could be due to outbreaks of this pathotype. This finding is in accordance with reports that showed that the presence of EIEC is principally caused by outbreaks (Beutin et al., 1997; Gordillo et al., 1992). During an outbreak, there are usually common sources of infection and the isolates are often genetically related (Beutin et al., 1997; Tenover et al., 1995). However, pulsed field gel electrophoresis analysis of EIEC isolates carried out during periods of high prevalence suggested polyclonal nature (Vieira et al., 2007). Although EIEC is the second most prevalent pathotype, EIEC was the least associated with diarrhea. The steady and significant decline in EIEC prevalence from March 2006 to December 2010 could be due to improved sanitary conditions and personal hygiene since EIEC is transmitted mainly by person-to-

person contact or food manipulation (Harris et al., 1985). Additionally, EIEC prevalence could be determinate by the presence of other pathogens since EIEC has a limited virulence (Faundez et al., 1988; Ramos et al., 2009), so this could be responsible of the pattern of prevalence showed. In contrast, *E. coli* Shigellae was least prevalent but had the highest associated risk for diarrhea. The public health impact of *E. coli* Shigellae may be high since very low doses appear to cause disease (DuPont et al., 1989).

Environmental factors such as rainfall could potentially affect the prevalence of some pathotypes. For example, ETEC is found frequently in water where it is thought to survive for long periods of time (Lothigius et al., 2010; Okeke, 2009; Qadri et al., 2005). This pathotype has also been implicated in the majority of water-borne outbreaks (Brüssow et al., 1992; Daniels et al., 2000; Huerta et al., 2000).Thus; water may be the main source of ETEC in these remote communities. Unlike ETEC, EIEC is more sensitive to environmental conditions (Nwachuku & Gerba, 2008; Rosak & Cowell, 1987). Another pathotype that can survive in water and retain its virulence is *E. coli* Shigellae (Khalil et al., 1998; Rahman et al., 1994); nevertheless, it existence probably depends on the presence of the other bacteria since *E. coli* Shigellae compete poorly with other bacteria (Alcoba-Flórez et al., 2005).

According to these results ETEC carrying LT toxin genes were the most common between November 2004 and March 2008. These results differ from those previously published in which the most prevalent ETEC was ETEC-ST (Albert et al., 1999; Qadri et al., 2000; Shaheen et al., 2004). However, ETEC carrying ST toxin genes were the most prevalent after March 2008. It may be that ETEC-LT infections, unlike ETEC-ST infections, induce protective immunity so that infection is restricted to the first exposure (Glenn et al., 2008; Gupta et al., 2008). Additionally, ETEC-ST was strongly associated

with diarrhea as reported in previous studios (Albert et al., 1999; Qadri et al., 2000; Shaheen et al., 2004). It is likely that ETEC-ST cause more severe disease than ETEC-LT (Gupta et al., 2008; Qadri et al., 2005).

Atypical EPEC may be more common than typical EPEC in developing countries (Afset et al., 2004; Ochoa et al., 2008; Scaletsky et al., 2009; Trabulsi et al., 2002). Low EPEC prevalence from 2003 to 2010 may be due our detection of only typical EPEC. In the present study, EPEC isolates were detected by the presence of *bfp* and therefore, we may have missed atypical EPEC which lack this gene (Kaper et al., 2004; Trabulsi et al., 2002). Although EPEC prevalence is low, this pathotype had a strong and significant association with diarrhea.

In conclusion, the present study suggests that ETEC is the dominant *E. coli* pathotype in remote communities of northwestern Ecuador. The apparent dominance of EIEC in a previous study seems to be the result of a temporary increase in EIEC prevalence. Other studies have found year to year variation in the prevalence of other enteric pathogens such as types of *E. coli* Shigellae (Kirnpal-Kaur et al., 2011) , or types of ETEC (Qadri et al., 2000; Rao et al., 2003; Shaheen et al., 2004) in diarrheal samples. Other studies have looked at the variation in the prevalence of bacterial pathogens belonging to different species also in diarrheal samples (Kanta et al., 2008; Lee et al., 2002). To our knowledge this is the first study where the prevalence of four *E. coli* pathotypes was analyzed over time in symptomatic and asymptomatic individuals.

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7. REFERENCES

1. Afset, J., Bevanger, L., Romundstad, P., & Bergh, K. (2004). Association of atypical enteropathogenic *Escherichia coli* (EPEC) with prolonged diarrhoea. *J. Med. Microbiol*. 53: 1137-1144.

2. Albert, M., Faruque, A., Faruque, S., Sack, R., & Mahalanabis, D. (1999). Case-control study of enteropathogens associated with childhood diarrhea in Dhaka, Bangladesh. *J. Clin. Microbiol*. 37 (11): 3458-3464.

3. Alcoba-Flórez, J., Pérez-Roth, E., González-Linares, S., & Méndez-Álvarez, S. (2005). Outbreak of *Shigella sonnei* in a rural hotel in La Gomera Canary Islands, Spain. *Int. Microbiol*. 8 (2): 133-136.

4. Beutin, L., Gleier, K., Kontny, I., Echeverria, P., & Scheutz, F. (1997). Origin and characteristics of enteroinvasive strains of *Escherichia coli* (EIEC) isolated in Germany. *Epidemiol. Infect*. 118 (3): 199-205.

5. Brüssow, H., Sidoti, J., Link, H., Hoang, Y., Barclay, D., Dirren, H., & Freire, W. (1990). Age-specific prevalence of antibody to enterotoxigenic *Escherichia coli* in Ecuadorian and German children. *J. Infect. Dis*. 162 (4): 974-977.

6. Brüssow, H., Rahim, H., & Freire, W. (1992). Epidemiological analysis of serologically determined Rotavirus and enterotoxigenic *Escherichia coli* infections in Ecuadorian children. *J. Clin. Microbiol*. 30 (6): 1585-1587.

7. Daniels, N., Neimann, J., Karpati, A., Parashar, U., Greene, K., Wells, J., Srivastava, A., Tauxe, R., Mintz, E., & Quick, R. (2000). Traveler´s diarrhea at sea: three outbreaks of waterborne enterotoxigenic *Escherichia coli* on cruise ships. *J. Infect. Dis*. 181 (4): 1491-1495.

8. DuPont, H., Levine, M., Hornick, R., & Formal, S. (1989). Inoculum size in shigellosis and implications for expected mode of transmission. *J. Infect. Dis*. 159 (6): 1126-1128.

9. Faundez, G., Figueroa, G., Troncoso, M., & Cabello, F. (1988). Characterization of enteroinvasive *Escherichia coli* strains isolated from children with diarrhea in Chile. *J. Clin. Microbiol*. 26 (5): 928-932.

10. Glenn, G., Francis, D., & Danielsen, E. (2009). Toxin-mediated effects on the innate mucosal defenses: implications for enteric vaccines. *Infect. Immun*. 77 (12): 5206-5215.

11. Gordillo, M., Reeve, G., Pappas, J., Mathewson, J., DuPont, H., & Murray, B. (1992). Molecular characterization of strains of enteroinvasive *Escherichia coli* O143, including isolates from a large outbreak in Houston, Texas. *J. Clin. Microbiol*.30 (4): 889-893.

12. Guerrant, R., Van Gilder, T., Steiner, T., Thielman, N., Slutsker, L., Tauxe, R., Hennessy, T., Griffin, P., DuPont, H., Brandley, R., Tarr, P., Neil, M., Nachamkin, I., Reller, L., Osterholm, M., Bennish, M., & Pickring, L. (2001). Practice guidelines for the management of infectious diarrhea. *Clin, Infect. Dis*. 32 (3): 331-351.

13. Gupta, S., Keck, J., Ram, P., Crump, J., Miller, M., & Mintz, E. (2008). Part III. Analysis of data gaps pertaining to enterotoxigenic *Escherichia coli* infections in low and medium human development index countries, 1984-2005. *Epidemiol. Infect.* 136 (6): 721-738.

14. Harris, J., Mariano, J., Wells J., Panye, B., Donnell, H., & Cohen, M. (1985). Person to person transmission in an outbreak of enteroinvasive *Escherichia coli*. *Am. J. Epidemiol*. 122 (2): 245-252.

15. Herrera, A., Espinosa, B., Nuñez, G., Espinoza, N., Maves, R., & Martin, G. (2010). The effect of preparation of cebiche on the survival of enterotoxigenic *Escherichia coli, Aeromonas hydrophila*, and *Vibrio parahaemolyticus*. *J. Travel Med*. 17 (6): 395-399.

16. Horvitz, D. G., & Thompson, D. J. (1952). A generalization of sampling without replacement from a finite universe. *J. Am. Statis. Ass.*47 (260): 663-685.

17. Huerta, M., Grotto, I., Gdalevich, M., Mimouni, D., Gavrieli, B., Yavzori, M., Cohen, D., & Shpilberg, O. (2000). A waterborne outbreak of gastroenteritis in the Golan height due to enterotoxigenic *Escherichia coli*. *Infection*. 28 (5): 267-271.

18. Hunter, P. (2003). Drinking water and diarrhoeal disease due to *Escherichia coli*. *J. Water Health*. 01.2: 65-72.

19. Jain, S., Chen, L., Dechet, A., Hertz, A., Brus, D., Hanley, K., Wilson, B., Frank, J., Greene, K., Parsons, M., Bopp, C., Todd, R., Hoekstra, M., Mintz, E., & Ram, P. (2008). An outbreak of enterotoxigenic *Escherichia coli* associated with sushi restaurant in Nevada, 2004. *Clin. Infect. Dis*. 47 (1): 1-7.

20. Kanta, S., Khuntia, H., Nanda, P., Satapathy, C., Nayak, S., Sarangi, A., Sahoo, N., Pattnaik, S., Chhotray, G., & Pal, B. (2008). Incidence of bacterial enterophatogens among hospitalized diarrhea patients from Orissa, India. *Jpn. J. Infect. Dis*. 61: 350-355.

21. Kaper, J., Nataro, J., & Mobley, H. (2004). Pathogenic *Escherichia coli*. *Nature Rev*. 2: 123-140.

22. Khalil, K., Khab, S., Mazhar, K., Kaijser, B., & Lindblom, G. (1998). Occurrence and susceptibility to antibiotics of *Shigella* species in stools of hospitalized children with bloody diarrhea in Pakistan. *Am. J. Trop. Med. Hyg*. 58 (6): 800-803.

23. Kirnpal-Kaur, B., Chandra, S., Zainy, Z., & Rahman, R. (2011). A 9-year study of shigellosis in northeast Malaysia: antimicrobial susceptibility and shifting species dominance. *J. Public Health*. 19: 231-236.

24. Kumar, H., Parvathi, A., Karunasagar, A., & Karunasagar, I. (2005). Prevalence and antibiotic resistance of *Escherichia coli* in tropical seafood. *World J. Microbiol. Biotechnol.* 21: 619-623.

25. Lan, R., Lumb, B., Ryan, D., & Reeves, P. (2001). Molecular evolution of large virulence plasmid in *Shigella* clones and enteroinvasive *Escherichia coli*. *Infect. Immun*. 69 (10): 6303-6309.

26. Lee, W., Puchucheary, S., & Path, F. (2002). Bacterial enteropathogens isolated in childhood diarrhoea in Kuala Lumpur-The changing trend. *Med. J. Malaysia*. 57 (1): 24-30.

27. Levine, M. M. (1987). *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic and enteroadherent. *J. Infect. Dis*. 155 (3): 377-379.

28. Lothigius, Å., Sjöling, Å., Svennerholm, A. M., & Bölin, I. (2010). Survival and gene expression of enterotoxigenic *Escherichia coli* during long-term incubation in sea water and freshwater. *J. Appl. Microbiol.* 108: 1441-1449.

29. MacDonald, K., Eidson, M., Strohmeyer, C., Levy, M., Wells, J., Puhr, N., Wachsmuth, K., Hargrett, N., & Cohen, M. (1985). A multistate outbreak of gastrointestinal illness caused by enterotoxigenic *Escherichia coli* in imported semisoft cheese. *J. Infect. Dis.* 151 (4): 716-720.

30. Nataro, J. P., & Kaper, J. B. (1998). Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev*. 11(1): 142-201.

31. Nwachuku, N., & Gerba, C. (2008). Occurrence and persistence of *Escherichia* coli 0157:H7 in water. *Rev. Environ. Sci. Biotechnol.* 7 (3):267-273.

32. Ochoa, T., Barletta, F., Contreras, C., & Mercado E. (2008). New insights into the epidemiology of enteropathogenic *Escherichia coli* infection. *Trans. R. Soc. Trop. Med. Hyg*. 102 (9): 852-856.

33. Okeke, I. (2009). Diarrheagenic *Escherichia coli* in Sub-Saharan Africa: status, uncertainties and necessities. *J. Infect. Dev. Ctries*. 3 (11): 817-842.

34. Qadri, F., Kumar, S., Farunque, A., Fuchs, G., Albert, M., Sack, R., & Svennerholm, A. M. (2000). Prevalence of toxin types and colonization factors in enterotoxigenic *Escherichia coli* isolated during a 2-year period from diarrheal patients in Bangladesh. *J. Clin. Microbiol.* 38 (1): 27-31.

35. Qadri, F., Svennerholm, A. M., Faruque, A., & Sack, R. (2005). Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features treatment, and prevention. *Clin. Microbiol. Rev*. 18 (3). 465-483.

36. Rahman, I., Shahamat, M., Kirchman, P., Russek-Cohen, E., & Colwell, R. (1994). Methionine uptake and cytopathogenicity of viable but noculturable *Shigella dysenteriae* Type 1. *Appl. Environ. Microbiol*. 60 (10): 3573-3578.

37. Ramos, A., Gonçalves, L., & Baquerizo, M. (2009). Enteroinvasive *Escherichia coli* vs. *Shigella flexneri*: how different patterns of gene expression affect virulence. *FEMS Microbiol. Lett.* 301: 156-163.

38. Rao, M., Abu-Elyazeed, R., Savarino, S., Naficy, A., Wierzba, T., Abdel-Messih, I., Shaheen, H., Frenck, R., Svennerholm, A. M., & Clemens, J. (2003). High disease burden of diarrhea due to enterotoxigenic *Escherichia coli* among rural Egyptian infants and young children. *J. Clin. Microbiol*. 41 (10): 4862-4864.

39. Roszak, D., & Colwell, R. (1987). Survival strategies of bacteria in the natural environment. *Microbiol Rev.* 51 (3): 365-379.

40. Sasakawa, C. (1997). Early states of *Shigella* interaction with host cells. *J. Infect. Chemother*. 3 (2): 63-72.

41. Scaletsky, I., Aranda, K., Souza T., Silva, N., & Morais, M. (2009). Evidence of pathogenic subgroups among atypical enteropathogenic *Escherichia coli* strains. *J. Clin. Microbiol.* 47 (11): 3756-3759.

42. Shaheen, H., Khalil, S., Rao, M., Abu Elyazeed, R., Wierzba, T., Peruski, L., Putnam, S., Navarro, A., Morsy, B., Cravioto, A., Clemens, J., Svennerholm, A. M., & Savarino, S. (2004). Phenotypic profiles of enterotoxigenic *Escherichia coli* associated with early childhood diarrhea in rural Egypt. *J. Clin. Microbiol.* 42 (12): 5588-5595.

43. Talukder, K., Aminul, M., Khajanchi, B., Dutta, D., Islam, Z., Safa, A., Alam, K., Hossain, A., Nair, G., & Sack, D., (2003). Temporal shifts in the dominance of serotypes of *Shigella dysenteriae* from 1999 to 2002 in Dhaka, Bangladesh. *J. Clin. Microbiol.* 41 (11): 5053-5058.

44. Tenover, F., Arbeit, R., Goering, R., Mickelsen, R., Murray, B., Persing, D., & Swaminathan, B. (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol*. 33 (9): 2233-2239.

45. Tornieporth, N., John, J., Salgado, K., De Jesus, P., Latham, E., Melo, M. C., Gunzburg, S. T., & Riley, L. W. (1995). Differentiation of pathogenic *Escherichia coli* strains in Brazilian children by PCR. *J. Clin. Microbiol*. 33 (5): 1371-1374.

46. Trabulsi, L., Keller, R., & Tardelli, T. (2002). Typical and atypical enteropathogenic *Escherichia coli*. *Emerg. Infect. Dis*. 8 (5): 508-513.

47. Vieira, N., Bates, S., Solberg, O., Ponce, K., Howsmon, R., Cevallos, W., Trueba, G., Riley, L., & Eisenberg, J. (2007). High prevalence of enteroinvasive

Escherichia coli isolated in a remote region of northern coastal Ecuador. *Am. J. Trop. Med. Hyg*. 76 (3): 528-533.

48. Whitten, N. (1965). Class kinship and power in an Ecuadorian town: the negroes of San Lorenzo. Stanford, CA: Standford University Press.

49. World Health Organization, (2010). Diarrhea disease. Available at: http://www.who.int/topics/diarrhoea/en/. [Accessed August 15, 2010].

50. Yang, J., Nie, H., Chen, L., Zhang, X., Yang, F., Xu, X., Zhu, Y., Yu, L., & Jim, Q. (2007). Revising the molecular evolutionary history of *Shigella* spp. *J. Mol. Evol*. 64 (1): 71-79.

PART III

TABLES AND FIGURES

Table 1. Prevalence of *Escherichia coli* pathotypes and *E. coli* Shigellae in 16 remote communities in northwestern Ecuador. N: number of samples containing *E. coli* pathotypes: ETEC (enterotoxigenic *Escherichia coli*), EPEC (enteropathogenic *Escherichia coli*), EIEC (enteroinvasive *Escherichia coli*). CI: 95 % confidence interval. Prevalence estimates are weighted according to the sampling fraction of all cases and controls. Prevalence of cases and controls was estimated based on total cases weighted and total control weighted respectively of all communities.

	***E. coli* Pathotypes**						
	ETEC				**EPEC**		
Cycles	**Tested (N) (cases-controls)**	**Positives (cases-controls)**	**Prevalence (%) (Cases-Controls)**	**[95 % CI]**	**Positives (cases-controls)**	**Prevalence (%) (Cases-Control)**	**[95 % CI]**
Aug 2003 – Jan 2004	298 (82-216)	1 (1-0)	0.05 (1.22 0.00)	[-0.65 0.76]	1 (0-1)	0.28 (0.0 0.28)	[-1.31 1.86]
Mar 2004 – Oct 2004	264 (65-199)	6(6-0)	0.30 (9.23 0.00)	[-0.70 1.30]	2 (0-2)	0.88 (0.00 0.88)	[-0.83 2.59]
Nov 2004 – Jul 2005	456 (101-355)	15 (10-5)	1.63 (9.90 1.38)	[-0.11 3.36]	3 (1-2)	0.25 (0.99 0.18)	[-0.43 0.93]
Aug 2005 – Mar 2006	505 (122-423)	9 (6-3)	0.76 (4.92 0.74)	[-0.24 1.76]	6 (6-0)	0.15 (4.92 0.00)	[-0.29 0.59]
May 2006 – Dec 2006	555 (134-421)	30 (16-14)	3.53 (11.94 3.17)	[1.48 5.57]	5 (2-3)	0.87 (1.49 0.84)	[-0.16 1.91]
Feb 2007 – Jul 2007	273 (74-199)	12 (6-6)	3.71 (8.12 3.54)	[0.41 7.01]	3 (3-0)	0.07 (4.05 0.00)	[-0.39 0.54]
Sep 2007 – Mar 2008	377 (92-285)	19 (14-5)	2.57 (15.21 2.25)	[-0.02 5.16]	2 (0-2)	1.28 (0.00 1.24)	[-0.56 3.12]
Dec 2008 – Nov 2009	748 (125-613)	16 (4-12)	2.19 (2.98 2.02)	[1.02 3.37]	4 (1-3)	0.54 (0.74 0.49)	[-0.05 1.12]
Jan 2010- Dec 2010	720 (111-609)	17 (10-7)	1.44 (9.00 1.14)	[0.46 2.43]	1 (1-0)	0.02 (0.90 0.00)	[-0.09 0.13]

Table 1. Prevalence of *Escherichia coli* pathotypes and *E. coli* Shigellae in 16 remote communities in northwestern Ecuador. Continuing…...

Cycles	*E. coli* Pathotypes						
	* EIEC				* *E. coli* Shigellae		
	Tested (N) (cases-controls)	Positives (cases-controls)	Prevalence (%) (cases-controls)	[95 % CI]	Positives (cases-controls)	Prevalence (%) (cases-controls)	[95 % CI]
Nov 2004 – Jul 2005	456 (101-355)	7 (2-5)	1.13 (1.98 0.68)	[-0.32 2.58]	4 (0-4)	0.48 (0.00 0.59)	[-0.47 1.42]
Aug 2005 – Mar 2006	505 (122-423)	26 (4-22)	4.44 (3.28 4.84)	[2.06 6.83]	10 (10-0)	0.25 (8.19 0.00)	[-0.33 0.82]
May 2006 – Dec 2006	555 (134-421)	16 (4-12)	2.35 (2.98 2.56)	[0.67 4.02]	17 (10-7)	1.28 (7.46 1.15)	[0.04 2.53]
Feb 2007 – Jul 2007	273 (74-199)	10 (3-7)	2.76 (4.05 3.26)	[-0.10 5.62]	6 (3-3)	1.67 (4.05 2.18)	[-0.57 3.91]
Sep 2007 – Mar 2008	377 (92-285)	8 (3-5)	1.97 (3.26 2.10)	[-0.30 4.24]	3 (2-1)	0.09 (2.17 0.04)	[-0.40 0.58]
Dec 2008 – Nov 2009	748 (125-613)	11 (2-9)	1.55 (1.49 1.48)	[0.56 2.54]	0	0	0
Jan 2010- Dec 2010	720 (111-609)	6 (0-6)	0.97 (0.00 0-98)	[0.21 1.74]	5 (3-2)	0.36 (2.70 0.32)	[-0.11 0.84]

Table 2. Prevalence of *Escherichia coli* pathotypes and *E. coli* Shigellae in Borbón from July 2005 to March 2010. N: number of samples containing *E. coli* pathotypes: ETEC (enterotoxigenic *Escherichia coli*), EPEC (enteropathogenic *Escherichia coli*), EIEC (enteroinvasive *Escherichia coli*). CI: 95% confidence intervals. Prevalence estimates are weighted according to the sampling fraction of all cases and controls using data from cycles 3 to 9 (November 2004 to December 2010). Prevalence of cases and controls was estimated based on total cases weighted and total control weighted respectively of all communities.

	***E. coli* Pathotypes**						
		ETEC			**EPEC**		
Month-Year	**Tested (N) (cases-controls)**	**Positives (cases-controls)**	**Prevalence (%) (cases-controls)**	**[95 % CI]**	**Positives (cases-controls)**	**Prevalence (%) (cases-controls)**	**[95 % CI]**
Jul 2005	154 (35-119)	7 (4-3)	2.1 (11.4 1.6)	[-0.7, 4.9]	1 (0-1)	0.5 (0.0 1.2)	[-0.9, 1.9]
Mar 2006	133 (30-103)	4 (3-1)	1. 6 (10.0 1.4)	[-1.0, 4.2]	0 (0-0)	0	0
Dec 2006	109 (28-81)	7 (3-4)	3.8 (14.3 3.4)	[-0.5, 8.0]	2 (0-2)	2.1 (0.0 2.0)	[-1.1, 5,3]
July 2007	66 (19-47)	2 (8-0)	0.3 (0.0 0.2)	[-1.5, 2.1]	1 (1-0)	0.1 (5.3 0.0)	[-1.1,1.4]
Mar 2008	98 (20-78)	4 (2-2)	2.3 (10 2.0)	[-1.3, 5.9]	0 (0-0)	0	0
Dec 2008 – Jan 2009	264 (65-199)	4 (0-4)	2.2 (0.0 2.0)	[0.1, 4.3]	1 (0-1)	0.5 (0.0 0.5)	[-0.5, 1.6]
Jan 2010- Mar 2010	236 (45-141)	13 (9-4)	3.0 (20.0 2.1)	[0.4, 5.6]	0 (0-0)	0	0

Table 2. Prevalence of *Escherichia coli* pathotypes and *E. coli* Shigellae in Borbón from July 2005 to March 2010. Continuing

Month-Year	*E. coli* Pathotypes						
		EIEC			*E. coli* Shigellae		
	Tested (N) (cases-controls)	Positives (cases-controls)	Prevalence (%) (cases-controls)	[95 % CI]	Positives (cases-controls)	Prevalence (%) (cases-controls)	[95 % CI]
Jul 2005	154 (35-119)	4 (0-4)	1.9 (0.0 2.9)	[-0,7, 4.5]	3 (0-3)	1.9 (0.0 2.7)	[-0.7, 4.5]
Mar 2006	133 (30-103)	19 (1-18)	18.5 (3.3 18.9)	[10.6, 26.4]	4 (4-0)	0.5 (13.3 0.0)	[-1.0, 2.0]
Dec 2006	109 (28-81)	3 (1-2)	2.2 (3.6 2.0)	[-1.1, 5.4]	5 (5-0)	0.6 (17.8 0,0)	[-1.1, 2.3]
July 2007	66 (19-47)	1 (0-1)	3.1 (0.0 3.1)	[-2.6, 8.8]	0	0	0
Mar 2008	98 (20-78)	3 (1-2)	2.1 (5.0 1.9)	[-1.3, 5.5]	0	0	0
Dec 2008 – Jan 2009	264 (65-199)	3 (0-3)	1.6 (0.0 1.5)	[-0.2, 3.4]	0	0	0
Jan 2010- Mar 2010	236 (45-141)	2 (0-2)	1.0 (0.0 1.0)	[-0.4, 2.4]	3 (1-2)	1.1 (2.2 1.1)	[-0.4, 2.5]

Table 3. Prevalence of enterotoxigenic *Escherichia coli* in 16 communities from November 2004-December 2010. ETEC: enterotoxigenic *Escherichia coli*, LT: heat-labile enterotoxin, ST: heat stable enterotoxin.CI: 95% confidence intervals. Prevalence estimates are weighted according to the sampling fraction of all cases and controls using data from cycles 3 to 9 (November 2003 to December 2010). Prevalence of cases and controls was estimated based on total cases weighted and total control weighted respectively of all communities. NA: not available.

	ETEC								
Cycles	**ETEC-LT**			**ETEC-ST**			**ETEC-LT-ST**		
	Positives (cases-controls)	**Prevalence (%) (cases controls)**	**[95 % CI]**	**Positives (cases-controls)**	**Prevalence (%) (cases controls)**	**[95 % CI]**	**Positives (cases-controls)**	**Prevalence (%) (cases controls)**	**[95 % CI]**
Nov 2004 – Jul 2005	12 (8-4)	1.29 (7.9 1.1)	[-0,26 2.83]	1 (1-0)	0.02 (0.9 0.0)	[-0.19 0.24]	2 (1-1)	NA	NA
Aug 2005 – Mar 2006	6 (4-2)	0.48 (3.3 0.7)	[-0.32 1.29]	3 (2-1)	0.28 (1.6 0.3)	[-0.33 0.88]	0 (0-0)	NA	NA
May 2006 – Dec 2006	21 (9-12)	2.94 (6.2 2.2)	[1.07 4.81]	6 (5-1)	0.33 (3.4 0.2)	[-0.31 0.97]	3 (2-1)	NA	NA
Feb 2007 – Jul 2007	6 (4-2)	2.61 (5,4 2.3)	[-0.18 5.39]	0	0	0	6 (2-4)	NA	NA
Sep 2007 – Mar 2008	9 (4-5)	2.34 (4.2 2.3)	[-1.13 4.82]	4 (4-0)	0.09 (4.2 0.0)	[-0.40 0.59]	6 (6-0)	NA	NA
Dec 2008 – Nov 2009	6 (2-4)	0.71 (1.5 0.6)	[0.04 1.39]	10 (2-8)	1.48 (1.5 1.4)	[0.51 2.46]	0	0	0
Jan 2010- Dec 2010	6 (3-3)	0.58 (2.7 0.5)	[-0.04 1.21]	6 (4-2)	0.61 (3.6 0.3)	[-0.03 1.25]	5 (3-2)	0.44 (2.7 0.3)	[-0.11 98]

Table 4. Pathogenicity of *Escherichia coli* pathotypes and *E. coli* Shigellae between August 2003 and December 2010. EIEC: enteroinvasive *Escherichia coli*, EPEC: enteropathogenic *Escherichia coli*, ETEC: enterotoxigenic *Escherichia coli*, LT: heat-labile enterotoxin, ST: heat stable enterotoxin. 95% CI: 95% confidence interval.

***E. coli* Pathotype**	**Risk Ratio**	**Bootstrapped Risk Ratio**	**Bootstrapped 95% CI**
EIEC*	1.15	1.17	(0.61, 1.96)
E. coli Shigellae*	6.90	7.44	(3.76, 13.69)
EPEC	3.46	3.83	(1.60, 8.06)
ETEC (LT and/or ST)	4.61	4.72	(3.11, 6.80)
ETEC LT only	3.70	3.84	(2.13, 6.46)
ETEC ST only	5.27	5.60	(2.68, 10.46)
ETEC LT+ST**	6.43	12.86	(0, 52.69)

***** Risk ratio for EIEC and *E. coli* Shigellae were calculated using data from November 2004 to December 2010.

** Risk ratio for ETEC LT + ST were calculated using data from December 2008 to December 2010.

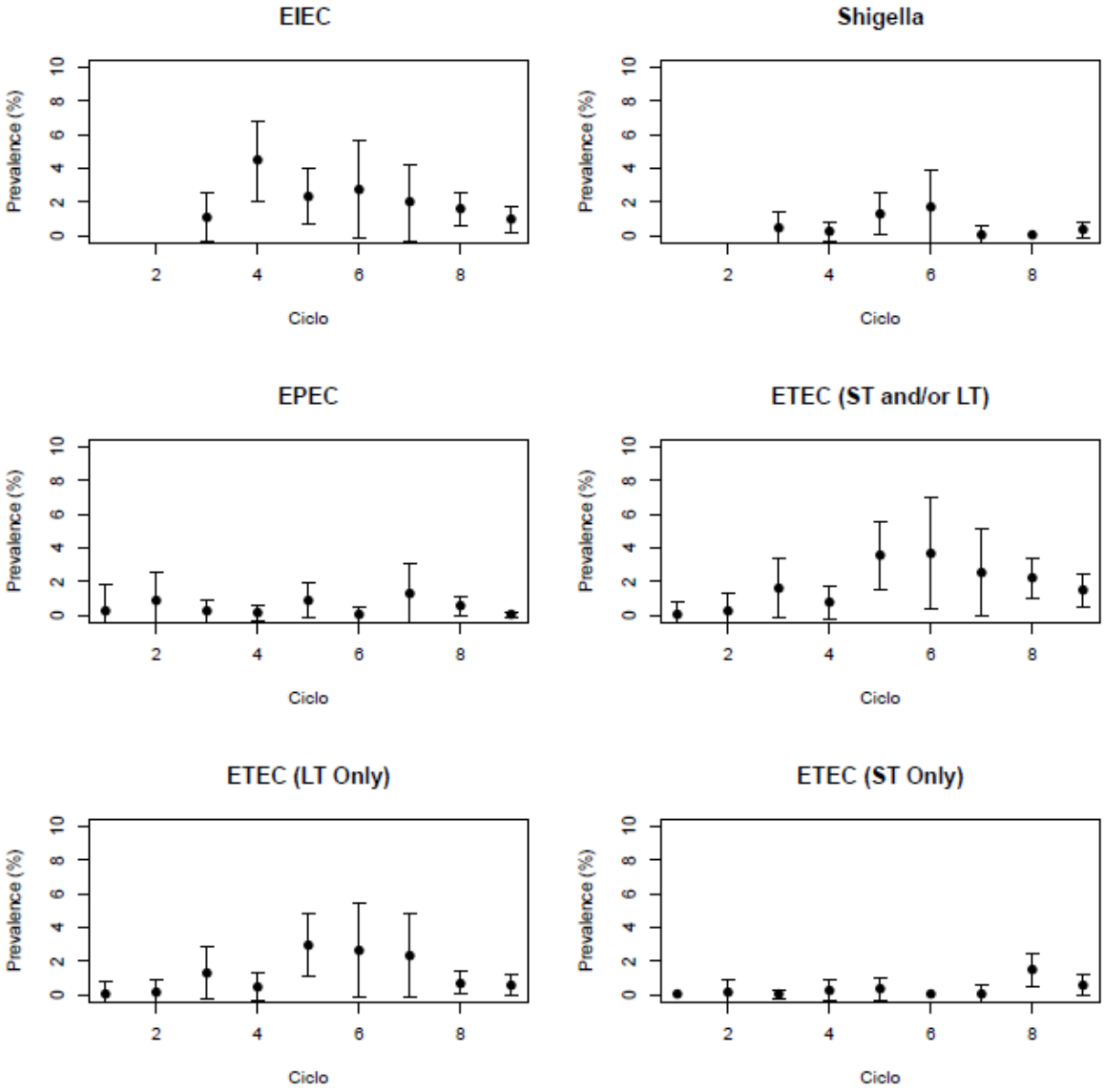

Figure 1.Temporal distribution in prevalence of *Escherichia coli* pathotypes and *E. coli* Shigellae from 2003 to 2010. Black lines depict the standard error of each prevalence estimate. EIEC: enteroinvasive *Escherichia coli*, ETEC: enterotoxigenic *Escherichia coli*, LT: heat-labile enterotoxin, ST: heat stable enterotoxin, EPEC: enteropathogenic *Escherichia coli*. Prevalence estimates are weighted according to the sampling fraction of all cases and controls.